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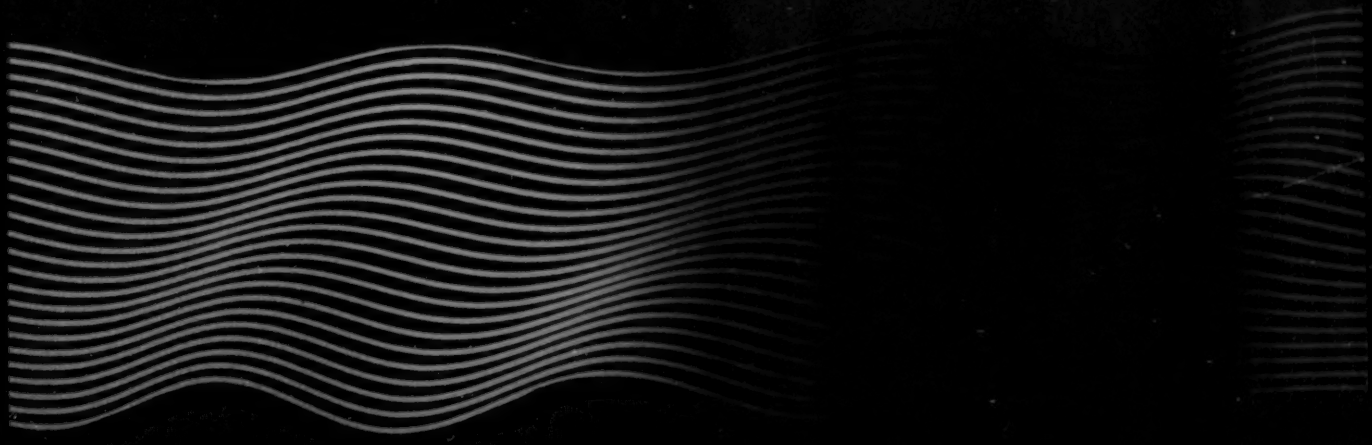

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A feedback model for the optimal management of renewable natural capital stocks

Leif K. Sandal and Stein I. Steinshamn

Abstract: The optimal management of the environment is becoming increasingly important with our growing awareness of the problems of loss of biodiversity and overconsumption of natural capital stocks. We used perturbation theory to provide simple feedback rules to compute optimal yields and depletion rates for many different types of renewable resources and to find the optimal steady state. The model is arbitrary in the state variable, stock, and quadratic in the control variable, yield. A quadratic objective function includes downward sloping demand or increasing marginal costs which are the most common sources of nonlinearities in economics. As a consequence of the nonlinearity, it is seen analytically that optimal stock levels may increase or decrease with an increased discount rate. The approach allows for managers to perform parameter analysis and to set yields even when the environmental system is subject to exogenous shocks. The feedback rules typify an adaptive approach to resource management and should prove useful in improving the management of the local and global commons.

Résumé : Au vu de l'accroissement de la sensibilisation aux problèmes de perte de biodiversité et de surconsommation des ressources naturelles capitales, il est de plus en plus important de gérer l'environnement de façon optimale. Nous avons utilisé la théorie des perturbations pour fournir des règles de rétroaction simples dans le but de calculer les rendements optimums et les taux d'épuisement de diverses ressources naturelles, ainsi que de déterminer l'état d'équilibre optimal. Le modèle est arbitraire pour la variable d'état, le stock, et quadratique pour la variable de contrôle, la production. Une fonction économique quadratique comporte une décroissance de la demande ou une augmentation du coût marginal, les causes de non-linéarité les plus fréquentes en économie. La non-linéarité permet de constater analytiquement que les niveaux optimums des stocks peuvent s'accroître ou décroître pour une augmentation du taux d'escompte. L'approche permet aux gestionnaires d'effectuer une analyse des paramètres et de fixer des objectifs de production même lorsque le système environnemental est soumis à un choc exogène. Les règles de la rétroaction caractérisent une approche d'adaptation à la gestion des ressources et devraient être utile pour l'amélioration de la gestion des patrimoines local et mondial.
[Traduit par la Rédaction]

Introduction

In recent years, there has been widespread overexploitation of renewable resources that has resulted in the collapse of important fish stocks such as Canada's northern Atlantic cod (*Gadhus morhua*) fishery (Hutchings and Myers 1994). The mismanagement of natural resources has led to concerns over loss of biodiversity (Fisher 1988) and overconsumption of the natural capital stock (Dasgupta 1990).

The problems in the environment are a function of many factors and include a lack of well-defined property rights (Scott 1985), subsidies for exploiting the natural capital stock (Feder 1977), market failures that arise from "thin" or absent markets for resources (Panayotou 1993), and an inadequate consideration for future generations (Howarth and Norgaard 1995). Even assuming that the owners and caretakers of renewable resources wish to maximize the net benefits over time, it is a difficult task to determine the desirable stock and rates of depletion and yields over time.

Many dynamic models that determine optimal yields assume that the control variable, the yield, enters into the optimization problem in a linear fashion. This leads to the result that yields should be zero if the current stock is less than the optimum and be at their maximum value if the stock is greater than the optimum. Despite the adoption of such a policy by Canada in the various moratoria in the Atlantic groundfish fisheries, so-called "bang–bang" strategies are unlikely to be desirable. This is because there are likely to be nonlinearities in the impact of yields on the net benefits derived from renewable resources.

The desire to have more realistic stock–yield trajectories has led various authors (Clark and Munro 1975; Conrad and Clark 1987; Clark 1990) to recommend feedback control laws or synthesized controls that can be derived from nonlinear models using numerical methods. The numerical methods approach is, however, often inexact and does not allow managers to examine the impact of different parameter values on optimal yields analytically. Deriving feedback controls is, nevertheless, important if managers of renewable resources are to determine the optimal yield as a function of the current value of the resource stock. In principle, a feedback rule allows one to determine the desirable total harvest in a fishery according to a relatively simple rule.

We developed an analytical rather than a numerical approach to setting a feedback rule for optimal yields of renewable natural capital stocks. The model presented here is

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deterministic, but, as it is a feedback model, it has the advantage that sporadic shocks or measurement errors still permit the yield trajectory to reach the optimal stock and can be readily applied by resource managers. Its contribution is that it provides a readily adaptable method for computing optimal yields and depletion rates for many different types of renewable resource stocks. It permits parameter analysis and thus is an adaptive approach (Hilborn and Walters 1992), which should prove useful in the management of natural resources. This approach also gives a simple criterion for when the optimal stock increases (decreases) as a result of an increased discount rate.

The following sections describe (*i*) the general model of a renewable resource, (*ii*) the approach in the case where there is no time dependence or discounting, (*iii*) the model with discounting, (*iv*) an example of practical use of the model, and (*v*) the conclusion.

General model

The general optimization problem for a resource manager wishing to maximize the net returns from a renewable stock over time is defined as

$$(1) \quad \max \int_0^T \Pi(h, x, t)\mathrm{d}t$$

subject to

$$(2) \quad \dot{x} = f(x,t) - h$$

$$x > \underline{x},\ 0 \le h \le \bar{h}$$

where Π denotes net benefit, h denotes yield, x denotes the resource stock, f denotes the natural growth or regeneration function of the stock, and t denotes time, while total time derivatives are denoted by dot notation. The functions Π and f can be completely general functions in x. The upper physical limit on harvest is $\bar{h}$, and $\underline{x} > 0$ is an exogeneously given barrier on the stock defined as the minimum safe stock based on ecological and biological criteria. This and all other notation is defined in the Appendix. The time horizon, T, can be both finite and infinite, athough we will focus on the infinite time horizon case.

The net benefit functions that we use to derive optimal yields can be specified as follows:

$$\Pi(h, x, t) = p(h, t)h - c(h, x, t)$$

where p represents a downward sloping inverse demand function that can be approximated as a linear function and c is a cost function that is at most quadratic in h but completely general in x. The model can also be modified to have p depend arbitrarily upon x. Given the nature of most natural capital stocks, however, the most realistic case is that costs are decreasing in x. Thus, we assume

$$p_h \le 0,\ p_{hh} = 0,\ c_h \ge 0,\ c_{hhh} = 0$$

where subscripts denote partial derivatives. Under these assumptions, Π is a quadratic function in h and a completely general function in x, which can be rewritten as follows

$$(3) \quad \Pi = \alpha(x, t) + \gamma(x, t)h - \Gamma(x, t)h^2$$

where α, γ, and Γ are assumed to be sufficiently regular (C^2) functions and nonnegative.

The maximization problem assumes that there is no excess capacity in obtaining the resource flow or yield. In other words, we look at the problem from the viewpoint of a sole owner. As the function Π is quite general, it may represent the producers' surplus, the consumers' surplus, the sum of the two, or any other preferred measure of social benefit. The necessary conditions for a solution of this optimization problem are derived in the Appendix. In the following section, we determine the optimal feedback rule for the yield in the simplest case where there is no explicit discounting or time dependence.

Autonomous model

Using the maximization problem and net benefit function specified in the previous section, we can examine the question of how to set optimal yields for natural capital stocks. The term α, which represents revenue or costs that are independent of the yield, is ignored in the following sections, as it does not affect the results. We first examine the simplest case of the model where there is no explicit time dependence or discounting such that future generations are considered as important as the present generation. Emphasis is also put on the case of zero discounting, as the main characteristics of the solution are clearly seen in this case. Furthermore, sensitivity analysis (see the Example section) shows that optimal paths are less sensitive to changes in the discount rate than they are to changes in the nonlinearity function Γ. This reconfirms the numerical results of Mendelssohn (1982). Net benefit is now a function of only x and h and is independent of time:

$$(4) \quad \Pi(h, x) = \gamma(x)h - \Gamma(x)h^2$$

To derive the optimal harvest as a function of the current stock level, it is useful to define the sustainable rent, $S(x)$:

$$S(x) \equiv \Pi(f(x), x) = \gamma(x)f(x) - \Gamma(x)f(x)^2$$

S is interpreted as the net benefit that accrues from any stock level, given that the stock is fixed, and only the surplus is harvested such that $h = f(x)$.

In the Appendix (eq. A7), optimal harvest is derived and is given by

$$(5) \quad h(x) = f(x) \pm \left(\frac{P_0 - S(x)}{\Gamma(x)}\right)^{1/2}$$

where P_0 is a constant that depends on the time horizon. P_0 can be interpreted as the Hamiltonian from optimal control theory, and it is the total rate of increase of total assets (see Clark 1990).

Equation 5 provides all of the information required to determine the optimal yield from renewable resources. It follows that the immediate change in the stock from applying the optimal yield policy is

$$(6) \quad \dot{x} \mp \left(\frac{P_0 - S(x)}{\Gamma(x)}\right)^{1/2}$$

where the minus should be chosen in eq. 5 whenever the stock level is below the optimal equilibrium, making $\dot{x} > 0$.

We can now examine the infinite time horizon problem. In this case, P_0 should be set equal to max(S), i.e., the global

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maximum of the S-curve corresponding to the stock level x^* where $\max(S) \equiv S(x^*)$. As P_0 can be interpreted as the Hamiltonian, an optimal choice of P_0 corresponds to the separatrix solution. The separatrices run through the equilibrium, x^*, and at the equilibrium $P_0 = S$. Maximizing P_0 is therefore equivalent to maximizing S implying $P_0 = S^*$.

The difference between P_0 and S has an important interpretation. Denote this difference as the dynamic potential (D):

$$D \equiv P_0 - S(x)$$

which is valid for any constant P_0. Squaring both sides of eq. 6, we can rewrite D as

$$(7) \qquad D(x, \dot{x}) = \Gamma(x)\dot{x}^2$$

D can be interpreted as the cost of not being at the optimal equilibrium. It is the difference between the net benefit we obtain from keeping the stock at its current level compared with the optimal level. Alternatively, we can interpret D as the potential gain from moving from the current point to the optimal equilibrium.

At the optimum, $D = 0$ and there is no incentive to change the stock. S is the net benefit keeping the stock at its current level, D is the potential gain from changing the stock, and P_0 is the total rate of increase of assets or the sum of S and D. D will always be nonnegative, as can be observed from eq. 7. It can also be explained intuitively as follows. Define the net average value of harvesting as $\kappa = \Pi/h$. Under this definition, D can be rewritten as

$$(8) \qquad D(\dot{x}, x) = [\kappa(h, x) - \kappa(f(x), x)]\dot{x}$$

At any stock level, a deviation of harvest from f results in a change in the stock level equal to $\dot{x} \neq 0$ and a deviation in the average value from $\kappa(f, x)$. The difference in average value is equal to the term in square brackets in eq. 8. An increase in the stock level is always associated with a harvest less than f, which results in an average value higher than $\kappa(f, x)$ — assuming a downward sloping demand (and vice versa). Consequently, D is always the product of two positive terms, two negative terms, or is zero. Thus, D can be restated as the sum of the flow of net returns due to a deviation from the steady state and the value of the rate of increase in the stock level.

We have shown that with an infinite time horizon the optimal yield path is given by eq. 5 with $P_0 = S^*$. This also defines a lower limit on the constant P_0. If P_0 is lower than S^*, the optimal steady state, x^*, will never be reached. When $P_0 = S^*$, it takes indefinitely long to reach the optimal equilibrium because the optimal path is given by the separatrices (e.g., see Clark 1990 or Samuelson 1965). If the functions involved are not sufficiently regular, the separatrices may reach the steady state in finite time but will still represent the optimal path. With a shorter time horizon, $P_0 > S^*$. Movement will no longer be along the separatrices, and equilibrium will be reached in finite time. The control path will in this case be discontinuous at the optimum. A higher P_0 level than S^* may be explained by managers who are impatient and are not prepared to wait indefinitely long to reach the optimum. The accumulated net benefit over time, however, will in this case be less than with an infinite time horizon, and thus, there is a cost associated with impatience. There is also an upper limit on P_0. This is defined by $h = \bar{h}$ whenever $x > x^*$ and $h = 0$ whenever $x < x^*$. In other words, the upper limit on P_0 is defined by the bang–bang approach.

Using these results, we can show that for a given initial stock, x_0, and a given optimal stock, x^*, the time horizon corresponding to an optimal trajectory is given by

$$T(x_0, x^*, P_0) = \int_{x_0}^{x^*} \left(\frac{\Gamma(z)}{P_0 - S(z)} \, dz \right)^{1/2}$$

where $x_0 \leq x^*$. It is seen that T is monotonically decreasing in P_0. The case where $x_0 > x^*$ can be analyzed in the same manner. There is also an allowable region for T. The upper bound on T is infinity whereas the lower bound is given by

$$T^{\min}(x_0, x^*) = \int_{x_0}^{x^*} \frac{dz}{f(z)}$$

i.e., the time it takes for the stock to reach x^* when it grows naturally at the rate f.

It is important to note that the equilibria in the dynamic model are given by the S-curve. The stationary points on the S-curve correspond to equilibria in the differential equations as follows: a local minimum corresponds to a centre, a local maximum corresponds to a saddlepoint, and an inflection point corresponds to a cusp. This is illustrated in Fig. 1. A typical S-curve derived from a Schaefer-type model with downward sloping demand is shown in Fig. 1*a* (for a more thorough discussion of the Schaefer model, see Clark 1990). The corresponding phase-diagram is shown in Fig. 1*b*. It follows that the global maximum must be a saddlepoint. The saddlepoint is unstable, as there are only two paths going through the equilibrium (the separatrices) and only one of them is stable.

In the infinite time horizon case the feedback rule for setting the optimal yield is directly seen from eq. 5. For this case the optimal feedback rule can be rewritten

$$(9) \qquad h = f(x) + sgn(x - x^*) \left(\frac{S^* - S(x)}{\Gamma(x)} \right)^{1/2}$$

Sporadic shocks and disturbances are not a problem with this feedback rule. When there are exogeneous shocks and disturbances that push the path off the optimal path, the rule above will lead to an optimum by continuously aligning the path to the separatrix. This feedback mechanism works in such a way that for any stock level, we start at time zero and find the optimal path which is the separatrix between x and x^*. The past history of the system is not relevant in deciding the present harvest. The past history of harvest and stock level may, however, be useful for refinement of the functions f and Π over time.

The model presented here is continuous in time. In practice, decisions about harvest will be taken according to regulatory conventions and according to the availability of stock estimates for x. For the management of renewable resources, eq. 5 is all that is required to determine optimal yields from a stock. This also permits analysis of the stock dynamics between periods, which is important in adpative management. This can be done directly by solving eq. 6 numerically or analytically. Straightforward application of optimal control theory yields a more complicated system characterized by the equations

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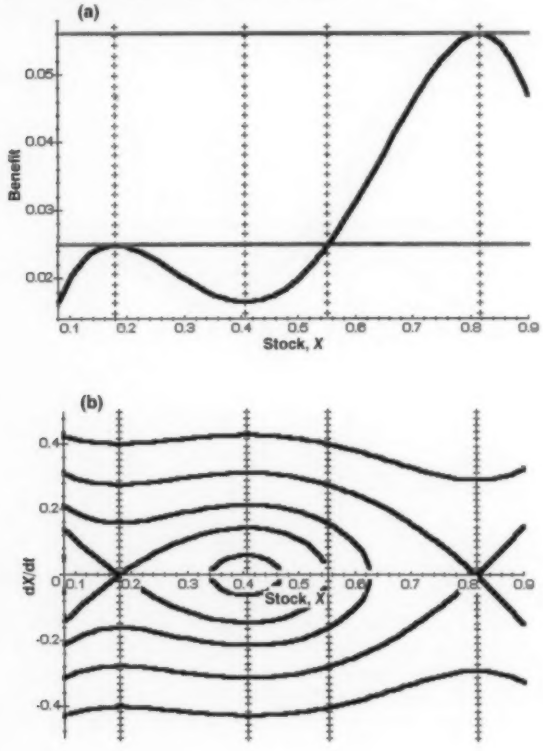
Fig. 1. (*a*) Representative *S*-curve and separatrix *P*-levels and (*b*) corresponding phaseplot with separatrices.



$$\dot{h} = \frac{\gamma' f + f'\gamma - 2\Gamma' hf + \Gamma' h^2 - 2f'\Gamma h}{2\Gamma}$$

$$\dot{x} = f - h$$

The approach described here also defines an economic barrier of the stock at the intersection between the separatrix coming from the left and the *f*-curve, as any control path above the *f*-curve in the phase-space is invalid. At stock levels smaller than this economic barrier, harvest is banned. If the economic barrier is higher than any biological barrier, the economic barrier will be binding. Otherwise, the biological barrier, $\underline{x}$, will be binding, thereby inducing a discontinuity at the barrier.

Model with discounting

We now examine the optimal feedback rule for the case where the net benefit is discounted at a rate δ:

$$\Pi(h, x, t) = e^{-\delta t}[\gamma(x)h - \Gamma(x)h^2]$$

In this section, perturbation theory is used to derive an analytical expression for optimal harvest (see the Appendix for details). The optimal harvest is given by

$$h(x, \delta) = f(x) - \frac{M}{2\Gamma}$$

and M is defined by

$$\dot{x} = \frac{M}{2\Gamma}$$

M can be expanded as follows:

$$M = M_0 + \delta M_1 + \delta^2 M_2 + O(\delta^3)$$

and M_0, M_1, and M_2 can be found as functions of P and S where

$$\tilde{S} = S + \delta \int_x^{x^*} \mu(z)dz$$

$$\tilde{P} = P_0 + \delta \int_x^{x^*} \mu(z)dz$$

and μ is defined as $\mu(x) = d\Pi/dh \mid_{h=f(x)}$. The function μ can be interpreted as the marginal value of a unit harvested when the stock is fixed such that the surplus is harvested. The integral $\int \mu dx$ can be interpreted as the value of the growth of the stock when it grows naturally. The finite integral $\int_x^{x^*} \mu dz$ is the value of future investment in the stock when it is allowed to grow naturally, and $\delta \int_x^{x^*} \mu dz$ is the alternative rate of return on this investment. At the optimal steady state, μ is equivalent to the adjoint (costate) variable.

To solve for the feedback rule, we need to determine $\tilde{P} = \tilde{P}_0 + \delta\tilde{P}_1 + \delta^2\tilde{P}_2 + \cdots$. This is done by setting $\tilde{P}_0 = S(x^*)$ where the optimal steady state, x^*, is found by solving

$$(10) \quad S'(x) = \delta\mu(x)$$

In the zero discounting case, $S' = 0$ at the optimal steady state. The interpretation of eq. 10 is that the value of a marginal unit of the stock should be the same whether it is left alone, S', or whether the marginal net benefit, μ, is invested and earns an alternative rate of return, δ. Equation 10 is valid for any nonlinear net benefit function and not only the quadratic referred to earlier. It is interesting to note that in nonlinear models, an increase in the discount rate may sometimes result in a higher optimal steady-state stock. This happens when $\mu < 0$ at the optimal steady state and will never be the case in linear single-species models. Typically, it happens when Γ is an increasing function in x. This point is thoroughly analyzed in Sandal and Steinshamn (1997). Hannesson (1983) has shown that in linear multispecies models, an increase in the discount rate may result in a higher standing stock of biomass.

The optimal steady state (h^*, x^*) is found sequentially by first solving eq. 10, giving x^*. In the case of multiple solutions the x-value yielding highest S is chosen. Then, h^* is found by $h^* = f(x^*)$. Using optimal control theory straightforwardly, two nonlinear equations with no immediate interpretation have to be solved:

$$\dot{h} = \frac{-\delta(\gamma - 2\Gamma h) + \gamma' f + f'\gamma - 2\Gamma' hf + \Gamma' h^2 - 2f'\Gamma h}{2\Gamma}$$

$$\dot{x} = f - h$$

The optimal harvest path is derived in the Appendix as

$$(11) \quad h = h_0 + \delta h_1$$

where h_0 is the optimal yield with zero discount rate given by eq. 9 and

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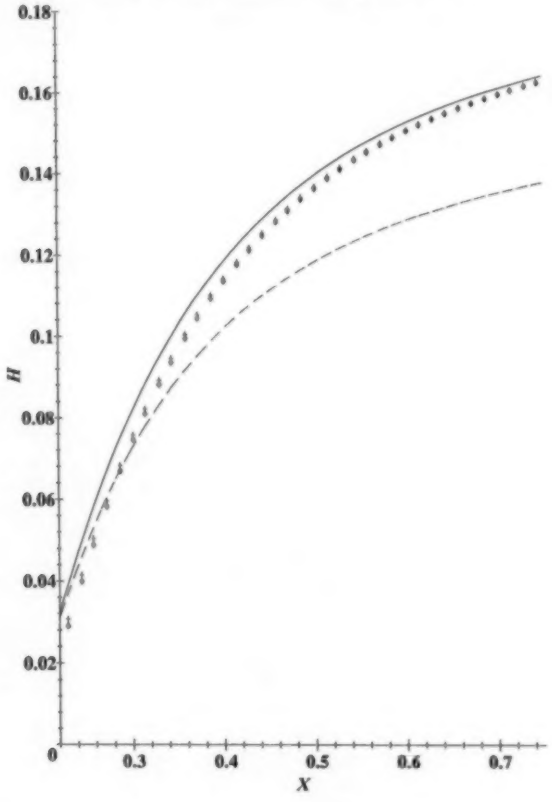
Fig. 2. Optimal harvest, H, calculated as a function of current stock level, X. Diamonds: $H = H_0$ with $G = 2.5$; plus signs: $H = H_0 + \rho H_1$ with $G = 2.5$ and $\rho = 0.1$; solid line: "exact" numerical solution with $G = 2.5$ and $\rho = 0.1$; broken line: $H = H_0$ with $G = 3$.



$$h_1 = \frac{\int_x^{x_0^*} [\gamma - 2\Gamma h_0]\mathrm{d}z}{4\Gamma(f - h_0)}$$

Given reasonable values for the discount rate δ, higher order corrections like $\delta^2 h_2$ can be neglected for all practical purposes. This shows that the optimal management of a renewable resource is also relatively straightforward in the case of a constant discount rate.

Example

This section illustrates practical use of the model based on a stylized example. The sensitivity of optimal paths to changes in the discount rate and changes in the degree of nonlinearity through Γ is also illustrated. The regeneration function of the natural capital stock is given by the logistic function

$$f(x) = rx\left(1 - \frac{x}{K}\right)$$

where r is the intrinsic growth rate and K is the carrying capacity of the stock. Further, Π is given by eq. 4 with

$$\gamma(x) = p_0 - \frac{a}{x}$$

where a is a constant. In this example also, Γ is set constant. The analysis is made dimensionless by rescaling the variables as follows:

$$X = \frac{x}{K},\ \theta = rt,\ H = \frac{h}{rK},\ \rho = \frac{\delta}{r},\ c_0 = \frac{a}{p_0 K},\ G = \frac{\Gamma r K}{p_0}$$

such that the maximization problem can be rewritten

$$\max \int e^{-\rho\theta}\left[\left(1 - \frac{c_0}{X}\right)H - GH^2\right]$$

subject to

$$\dot{X} = X(1 - X) - H$$

The numerical specification of the model is $G = 2.5$ or 3.0, $c_0 = 0.1$, and $\rho = 0$ or 0.1.

Optimal harvest paths calculated by eq. 9 for $\rho = 0$ and by eq. 11 for $\rho = 0.1$ are illustrated in Fig. 2. In addition the "exact" numerical solution calculated from the differential equations derived from optimal control theory for $\rho = 0.1$ is illustrated in the same figure. It is seen that these three curves are virtually the same. This shows that the difference between the numerical solution and the perturbation solution (eq. 11) can be neglected. Furthermore, the sensitivity of the optimal paths to a change in the discount rate from zero to 0.1 is so small that for reasonable values of ρ, eq. 9 can be used for all practical purposes. By reasonable values of ρ is meant $\rho < 1$ equivalent to $\delta < r$ in the case of a logistic model. These are also the values of ρ for which the perturbation scheme is valid. When $\rho > 1$, an alternative perturbation scheme has to be used.

Also illustrated in Fig. 2 is the sensitivity of the optimal paths to changes in the nonlinearity parameter G when $\rho = 0$. Two values of G are used, viz. 2.5 and 3. It is seen that the paths are much more sensitive to changes in G than they are to changes in the discount rate. In this model, G is proportional to the slope of the demand curve. In other words the demand curve for harvest is given by $p(h) = p_0 - \Gamma h$, which in the dimensionless model translates to $\hat{p}(H) = 1 - GH$. A consequence of this result is that it is more worthwile spending effort on estimating the elements of G than it is spending effort on finding the correct discount rate as long as $\rho < 1$.

Concluding remarks

Our well-being is directly related to the quality of our environment and natural resources. For a variety of reasons, renewable resources are often not managed optimally. An issue we raise here is how to determine the optimal yield from a renewable resource stock using a simple feedback rule. In contrast with the existing literature, we provide analytical expressions for optimal yields that can be derived from a general growth or regenerative function for a natural capital stock.

To derive the simple feedback rule, it is necessary to separate linear and nonlinear terms and separate the change in the stock from terms related to a steady state. The optimal yield is then derived analytically as a feedback control rule for the dynamic optimization problem outlined in eqs. 1 and 2. With zero discounting the optimal feedback rule is given by eq. 5 for an arbitrary time horizon and by eq. 9 for the more special

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case of an infinite time horizon. With positive discounting the optimal feedback rule is given by eq. 11. The optimal steady state for a completely general version of the problem given by eqs. 1 and 2, i.e., where Π is a general function in h, is given by eq. 10.

An important feature of this method is that it permits us to reach the optimal stock even if there are sporadic shocks or disturbances in the economic or ecological systems. The approach also lends itself to parameter analysis which allows resource managers to examine different scenarios when faced with uncertainty and calculate the dynamics of the stock between periods. Thus, it represents an adaptive approach to resource and environmental management. The general method should enable managers to set optimal yields for natural capital stocks and improve the use of local and global environments.

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Appendix

Alphabetical list of symbols and definitions

c	Cost function
$D = \Gamma \dot{x}^2$	Dynamic potential
f	Regeneration function of the stock
h	Yield
$\bar{h}$	Maximum yield
$\mathcal{H}$	Hamiltonian
M_n	Perturbation terms
p	Inverse demand function
$P \equiv S + D$	
P_0	Constant representing the Hamiltonian in the autonomous case
$S \equiv \Pi(f(x), x)$	Sustainable economic rent
t	Time
T	Time horizon
x	Renewable capital stock
$x^* = \arg\max(S)$	Optimal steady state stock level
$\underline{x}$	Minimum barrier on the stock
γ	Linear term in net benefit
δ	Discount rate
κ	Net average value of harvest
$\mu \equiv \left.\frac{d\Pi}{dh}\right\|_{h=f}$	
Γ	Quadratic term in net benefit
Π	Net benefit

Derivation of optimal rules

We derive the necessary conditions for an optimal solution in the case of general time dependence. Zero discounting and constant discounting are derived as special cases of the general rule.

General nonautonomous model

First, we apply the result from optimal control theory that

$$\text{(A1)} \quad \frac{d\mathcal{H}}{dt} = \frac{\partial \mathcal{H}}{\partial t}$$

where $\mathcal{H}$ is the Hamiltonian and, from the maximum principle, $\lambda = \partial\Pi/\partial h$. The optimal Hamiltonian in the case of the objective function given by eq. 3 is

$$\text{(A2)} \quad \mathcal{H} = \Pi(h, x, t) + \frac{\partial \Pi}{\partial h}\dot{x} = \alpha + \gamma f - \Gamma f^2 + \Gamma \dot{x}^2 = S + D \equiv P$$

where $S \equiv \alpha + \gamma f - \Gamma f^2$ and $D \equiv \Gamma \dot{x}^2$. Note that $d\mathcal{H}/dt = dP/dt$ but $\partial\mathcal{H}/\partial t \neq \partial P/\partial t$ because $P = P(\dot{x}, x, t)$ whereas $\mathcal{H} = \mathcal{H}(h, x, t, \lambda)$. Further,

$$\text{(A3)} \quad \frac{\partial \mathcal{H}}{\partial t} = \frac{\partial \Pi}{\partial t}(f - \dot{x}, x, t) = \frac{\partial}{\partial t}(P - 2D) - \frac{\partial \mu}{\partial t}\dot{x}$$

where $\mu \equiv \left.\partial\Pi/\partial h\right|_{h=f}$. Combining eqs. A1, A2, and A3, we get

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$$\frac{dP}{dt} = \frac{\partial P}{\partial t} - 2\frac{\partial D}{\partial t} - \frac{\partial \mu}{\partial t}\dot{x}$$

which can be rewritten

$$\text{(A4)} \quad \frac{\partial}{\partial \tau}\tilde{P} = -2\frac{\partial D}{\partial t}$$

where $\tilde{P} \equiv P - \int_x^{x_T} \frac{\partial}{\partial t}\mu(z, t)dz$, and the indirect time derivative is defined by

$$\frac{\partial}{\partial \tau} \equiv \frac{d}{dt} - \frac{\partial}{\partial t} = \dot{x}\frac{\partial}{\partial x} + \ddot{x}\frac{\partial}{\partial \dot{x}}$$

Equation A4 contains the optimal condition for general time dependence from which optimal conditions for all other kinds of time dependence can be derived as special cases. Note that the indirect time derivative can also be written

$$\frac{\partial}{\partial \tau} = \dot{x}\frac{d}{dx}$$

The result in eq. A4 can therefore alternatively be written

$$\dot{x}\frac{d\tilde{P}}{dx} = -2\frac{\partial \Gamma}{\partial t}\dot{x}^2$$

For a nonstatic stock, therefore, an optimal policy must in general confirm to the rule

$$\text{(A5)} \quad \frac{d\tilde{P}}{dx} = -\frac{\partial}{\partial t}M$$

where $M = M(x, \dot{x}, t) = 2\Gamma(x, t)\dot{x}$ and

$$\text{(A6)} \quad M^2 = 4\Gamma D = 4\Gamma(\tilde{P} - \tilde{S}) = 4\Gamma(P - S)$$

$\tilde{S}$ is defined accordingly to $\tilde{P}$ as $\tilde{S} \equiv S - \int_x^{x_T} \frac{\partial}{\partial t}\mu(z, t)dz$. These results are applied below.

Special case 1: autonomous model

In the case of zero discounting and no other explicit time dependence, optimal harvest is found by first noting that $\tilde{P} = P_0$ where P_0 is a constant because all partial derivatives with respect to time are zero. Substituting this into eq. A2 together with $\dot{x} = f - h$ and solving algebraically for h, we obtain

$$\text{(A7)} \quad h = f \pm \left(\frac{P_0 - S}{\Gamma}\right)^{1/2}$$

Thus for the autonomous model, it is straightforward to find an analytical expression for optimal harvest. The derivation of the constant P_0 is determined in the text.

Special case 2: model with discounting

From eq. A5, it is seen that

$$\text{(A8)} \quad \frac{d}{dx}\tilde{P} = \delta M$$

Equations A6 and A8 are now our basic equations. $\tilde{P}$ and M are defined according to the following perturbation scheme:

$$P = P_0 + \delta P_1 + \delta^2 P_2 + \cdots$$

$$\tilde{P} = P_0 + \delta(P_1 - \int_{x_T}^{x} \mu dz) + \delta^2 P_2 + \cdots, \quad \tilde{P}_n = P_n \; \forall \; n \neq 1$$

$$M = M_0 + \delta M_1 + \delta^2 M_2 + \cdots$$

This scheme is substituted into eqs. A6 and A8. With the definition $\tilde{S} = S + \delta\int_a^x \mu dz$, we have from eq. A6

$$(M^2)_0 = 4\Gamma(P_0 - S) = M_0^2$$

$$(M^2)_1 = 4\Gamma P_1 = 2M_0M_1$$

$$(M^2)_2 = 4\Gamma P_2 = 2M_0M_2 + M_1^2$$

$$(M^2)_n = 4\Gamma P_n = 2M_0M_n + \sum_{k=1}^{n-1} M_kM_{n-k}, \; n \geq 2$$

From eq. A8, we have

$$\tilde{P}_0' = P_0' = 0 \Rightarrow \tilde{P}_0 = P_0 = \text{constant}$$

$$\tilde{P}_1' = \left(P_1 - \int_{x_T}^{x} \mu dz\right)' = M_0 \Rightarrow P_1 = P_{1,0} + \int_a^x (M_0 + \mu)dz$$

$$P_{n+1} = P_{n+1,0} + \int_a^x M_n dz$$

where $P_{1,0}$, $P_{2,0}$, ... are constants. Combined, this yields

$$M_0^2 = 4\Gamma(P_0 - S), \; P_0 = \text{constant}$$

$$2M_0M_1 = 4\Gamma\left[P_1 + \int_a^x (M_0 + \mu)dz\right]$$

$$2M_0M_2 + M_1^2 = 4\Gamma\left[P_2 + \int_a^x M_1(z)dz\right], \text{ etc.}$$

To determine optimal harvest, the constants $P_{1,0}$, $P_{2,0}$, ... and a have to be determined. The optimal steady state is given by

$$\frac{d\tilde{P}}{dx} = S'(x) = \delta\mu(x)$$

solved for $x = x^*$. This yields from eq. A8

$$\text{(A9)} \quad \tilde{P}(x^*) = S(x^*)$$

Call the equilibrium with zero discount rate x_0^* and make an expansion about this. The new equilibrium is then $x^* = x_0^* + \delta x_1^* + \delta^2 x_2^* + \cdots$. Further,

$$S'(x^*) = S'(x_0^*) + \delta S''(x_0^*)x_1^* + \left[S''(x_0^*)x_2^* + \frac{1}{2}S'''(x_0^*)(x_1^*)^2\right]\delta^2 + \cdots$$

$$= \delta\mu(x^*) = \delta\mu(x_0^*) + \delta^2\mu'(x_0^*)x_1^* + \cdots$$

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Thus

$$\text{(A10)}\quad x_1^* = \frac{\mu(x_0^*)}{S''(x_0^*)},\ x_2^* = \frac{x_1^*}{S''(x_1^*)} \times\left[\mu'(x_0^*) - \frac{1}{2}x_1^* S'''(x_0^*)\right], \cdots$$

From eq. A10:

$$\begin{aligned}\tilde{P}(x^*) &= S(x^*) = S(x_0^*) + \delta S'(x_0^*)x_1^* \\ &\quad + \left[\frac{1}{2}S''(x_0^*)(x_1^*)^2 + S'(x_0^*)x_2^*\right]\delta^2 + \cdots \\ &= S(x_0^*) + \frac{1}{2}S''(x_0^*)(x_1^*)^2\delta^2 \\ &\quad + \left[\frac{1}{6}S'''(x_0^*)(x_1^*)^3 + S''(x_0^*)x_1^* x_2^*\right]\delta^3 + \cdots \\ &\equiv \tilde{P}_0 + \delta\tilde{P}_1(x_0^*) + \delta^2\tilde{P}_2(x_0^*) + \cdots\end{aligned}$$

This yields

$$\begin{aligned}\text{(A11)}\quad &\tilde{P}_0 = S(x_0^*) = P_0, \\ &\tilde{P}_1(x_0^*) = 0, \\ &\tilde{P}_2(x_0^*) = \frac{1}{2}S''(x_0^*)(x_1^*)^2, \\ &\tilde{P}_3(x_0^*) = S''(x_0^*)\,x_1^* x_2^* + \frac{1}{6}S'''(x_0^*)(x_1^*)^3, \cdots\end{aligned}$$

Equations A10 and A11 together determine the constant parameters P_0, P_1, P_2, ..., and a:

$$a = x_0^*,\ P_0 = \max\ S,\ P_1 = 0,\ P_2 = \left.\frac{\mu^2}{2S''}\right|_{x=x_0^*}, \cdots$$

The optimal harvest to order 3 can now be determined as follows:

$$h(x,\delta) = f(x) - \frac{M_0}{2\Gamma} - \delta\frac{M_1}{2\Gamma} - \delta^2\frac{M_2}{2\Gamma} + O(\delta^3)$$

Using the basic variables, this can be written as

$$h = h_0 + \delta h_1 + \delta^2 h_2 + O(\delta^3)$$

where h_0 is the optimal harvest with zero discount rate given by eq. A7 and

$$h_1 = \frac{\int_x^{x_0^*}[\gamma - 2\Gamma h_0]\mathrm{d}z}{4\Gamma(f - h_0)}$$

$$h_2 = \frac{1}{2\Gamma(f-h_0)}\left\{\Gamma h_1^2 - \int_x^{x_0^*} 2\Gamma h_1 \mathrm{d}z - \frac{\mu(x_0^*)^2}{2S''(x_0^*)}\right\}$$

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Gillnet selectivity for lake trout (*Salvelinus namaycush*) in Lake Superior

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Abstract: Gillnet selectivity for lake trout (*Salvelinus namaycush*) was estimated indirectly from catches in nets of 102-, 114-, 127-, 140-, and 152-mm stretch measure. Mesh selectivity was modeled as a nonlinear response surface that describes changes in the mean, standard deviation, and skewness of fish lengths across mesh sizes. Gillnet selectivity for lake trout was described by five parameters that explained 88% of the variation in wedged and entangled catches, 81% of the variation in wedged catches, and 82% of the variation in entangled catches. Combined catches of wedged and entangled lake trout were therefore described more parsimoniously than separate catches of wedged and entangled lake trout. Peak selectivity of wedged and entangled fish increased from 588 to 663 mm total length as mesh size increased from 102 to 152 mm, and relative selectivity peaked at a total length of 638 mm. The estimated lake trout population size–frequency indicated that gillnet catches were negatively biased toward both small and large lake trout. As a consequence of this bias, survival of Lake Superior lake trout across ages 9–11 was underestimated by about 20% when the catch curve was not adjusted for gillnet selectivity.

Résumé : Nous avons estimé indirectement la sélectivité des filets maillants pour le touladi (*Salvelinus namaycush*) à partir des captures faites au moyen de filets de 102, 114, 127, 140 et 152 mm (maille étirée). Nous avons modélisé la sélectivité du maillage sous forme de surface de réponse non linéaire qui décrit les changements dans la moyenne, l'écart-type et l'asymétrie des longueurs de poissons en fonction de la taille des mailles. Nous avons décrit la sélectivité des filets maillants pour le touladi à partir de cinq paramètres qui expliquent 88 % de la variation pour les captures maillées et emmêlées, 82 % de la variation pour les captures maillées et 85 % de celle des captures emmêlées. Nous avons donc décrit les captures combinées de poissons maillés et de poissons emmêlés de façon moins détaillée que les captures de maillés et emmêlés séparément. La sélectivité maximale pour les poissons maillés et emmêlés s'est accrue de 588 à 663 mm (longueur totale) pour une augmentation correspondante de la taille du maillage de 102 à 152 mm; la sélectivité relative a atteint un sommet à 638 mm de longueur totale. D'après l'estimation du rapport taille–fréquence de la population de touladi, il semblerait que les captures au filet maillant avaient un biais négatif pour les touladis de petite et de grande taille. En raison de ce biais, la survie du touladi de 9 à 11 ans dans le lac Supérieur était sous-estimée d'environ 20 % dans les cas où la courbe des captures ne tenait pas compte de la sélectivité des filets maillants.
[Traduit par la Rédaction]

Introduction

Gill nets are widely used for the commercial harvest and for the assessment of many fish populations, but are highly selective for certain sizes of fish. The size selectivity of a particular mesh size therefore needs to be known to effectively regulate its commercial use (Regier and Robson 1966). Gear selectivity, for example, can greatly affect the estimated allowable harvest for gillnet fisheries, which is crucial for managing these fisheries (Madenjian and Ryan 1995). The selectivity of a gillnet mesh size for a target species is normally determined from a series of different-sized gill nets that are simultaneously fished (Hamley 1975). The estimated selectivity of the various mesh sizes are then used to estimate the size composition of the target fish population, which is used to regulate the commercial fishery.

Various indirect methods have been proposed for estimating selectivity of gill nets to overcome the need for independent estimates of the size–frequency of the target population (Regier and Robson 1966; Hamley 1975). Central to such indirect methods of gillnet selectivity estimation is the assumption that the selectivity curves for all mesh sizes have the same shape and size (Baranov's geometrical similarity assumption; Hamley 1975). This assumption allows the computation of the underlying, but unknown, population size–frequency by scaling the selectivity of different mesh sizes to one size-class of fish (Type B curves) to the same height and then calculating the selectivity of a single mesh size (Type A curves).

Gillnet selectivity has not been estimated for lake trout

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(*Salvelinus namaycush*) in the Great Lakes, although Pycha (1962) estimated the relative efficiency of cotton and multifilament nylon gill nets for lake trout in Lake Superior. Previous estimates of gillnet selectivity for other species have usually been based on fish that were wedged in the meshes, but not for fish that were entangled in the meshes (Hamley 1975). Gillnet selectivity for wedged and entangled fish would be useful for managing a gillnet fishery, particularly when a substantial proportion of the fish caught in the gill net are entangled rather than wedged. Such gillnet fisheries for lake trout are still important in Lake Superior (Hansen et al. 1995), and gillnet selectivity estimates would therefore have practical value in managing these fisheries.

Total annual survival of a fish population can be estimated using the catch-curve method, but survival will be underestimated if catches used to construct the catch curve are not adjusted for gillnet selectivity (Regier and Robson 1966; Ricker 1975). The catch-curve method is used to estimate total annual mortality of lake trout caught in gillnet assessment fisheries in Lake Superior, but these estimates are acknowledged to overestimate mortality (underestimate survival) because of selectivity of the gill nets (Hansen et al. 1994).

In this study, we propose to model the selectivity of gill nets for lake trout to (1) determine if the shape and size of the selectivity curves are similar, as expected from the geometrical similarity assumption, (2) compare the selectivity curves for fish that were either wedged or entangled with the selectivity curves for fish that were wedged only and entangled only, and (3) compare survival estimates from catch curves derived from gillnet catches unadjusted for gillnet selectivity with survival estimates adjusted for gillnet selectivity.

Methods

We estimated the selectivity of five gillnet mesh sizes (102-, 114-, 127-, 140-, and 152-mm stretch measure) for lake trout using the nonlinear iterative least-squares method described by Helser et al. (1991, 1994). The residual least-squares errors of the estimated selectivity for the various mesh sizes were then used in a bootstrap procedure to estimate confidence intervals around estimates of relative selectivity and of the various size-classes of fish in the lake trout population in a refuge in western Lake Superior. We compared the relative selectivity of gill nets and population size–frequencies for fish that were wedged or entangled and for fish that were wedged only and entangled only. We also compared survival estimates from catch curves derived from gillnet catches unadjusted for gillnet selectivity with survival estimates adjusted for gillnet selectivity.

Gillnet catches

Nylon multifilament gill nets were set in Gull Island Refuge, near the Apostle Islands, Lake Superior, from 23 April through 9 May 1973. This location was chosen because a population of large, mostly native lake trout inhabited the area (Swanson and Swedberg 1980; Schram et al. 1995). Gill nets 3657.6 m in length were made up of eight 457.2-m sections, each of which was composed of five 91-m panels, 0.9144 m apart, with one panel each of 102-, 114-, 127-, 140-, and 152-mm stretch measure multifilament nylon twine. The net was 1.8288 m in height with a 1:2 hanging ratio. Net twine was 210/2 for the 102- to 140-mm meshes and 210/3 for the 152-mm mesh.

The net was lifted after 1 day on seven dates, but was lifted after 4 days on one date due to a storm. Lake trout were examined for mode of capture (wedged or entangled), measured (millimetres total length), and enumerated by mesh size. Fish were most often entangled by the mouthparts or teeth if they penetrated the mesh forward from the eyes, but were most often wedged if they penetrated the mesh past the eyes. Fish that penetrated a mesh so that the mesh encircled them between the eye and the dorsal fin were therefore considered wedged whereas others that were caught were considered entangled.

Girth measurements at the eye and at the dorsal fin were taken from a random sample of 170 fish to estimate the linear relationships between total length and girths at the eye and at the dorsal fin (Hamley 1975). We then plotted lengths of entangled fish against the perimeter of the mesh in which they were caught and compared these lengths with the maximum girth and eye girth linear relationships to determine which of the entangled lake trout could have been wedged in the same mesh. Any entangled lake trout that was caught in a mesh with a perimeter less than the upper 95% confidence interval of the maximum girth relationship or greater than the lower 95% confidence interval of the eye girth relationship was judged to have been capable of being wedged in that mesh. The 95% confidence intervals that we used for these comparisons were for individual observations, rather than for mean values, since we were comparing individual fish with the relationships (Draper and Smith 1981).

Model for estimating selectivity

We estimated gillnet selectivity indirectly because direct estimation requires knowledge of the true size structure of the fish population (Hamley 1975), and such data were unavailable. Helser et al. (1991, 1994) developed a method for indirectly fitting Type A and Type B selectivity curves simultaneously using a nonlinear iterative least-squares approach to estimate the response surface of gillnet selectivity as a function of both mesh size and fish size. The method improves on other approaches by simultaneously fitting Type A and Type B curves, by allowing assessment of overall model fit to the data, and by allowing evaluation of the reliability of parameter estimates (Helser et al. 1991; Millar 1992, 1995). The method can also be extended to estimate confidence intervals for the population size–frequency distribution (Helser et al. 1994). The method is based on an assumed skew-normal probability density function

$$s_{ij} = \frac{1}{\sigma_i (2\pi)^{1/2}} \exp^{-(l_j-\mu_i)^2/2\sigma_i^2} \left(1 - \frac{1}{2q_i\sigma_i^{3/2}} \left(\frac{(l_j-\mu_i)}{\sigma_i} - \frac{(l_j-\mu_i)^3}{3\sigma_i^3}\right)\right)$$

where s_{ij} = selectivity of mesh i for fish of size j, μ_i = mean size of fish caught (millimetres total length) in mesh size i, σ_i = standard deviation of the size of fish in mesh i, q_i = skewness coefficient of the size of fish in mesh i, and l_j = mean size of fish in size-class j. The moments of the probability density function, μ_i, σ_i, and q_i, are estimated as linear (or quadratic) functions of mesh size, m_i (millimetres stretch measure of mesh i). In our analysis, μ_i and σ_i were linearly related to m_i, and q_i was a constant:

$$\mu_i = \mu_0 + \mu_1 m_i$$

$$\sigma_i = \sigma_0 + \sigma_1 m_i$$

$$q_i = q_0$$

The estimating function

$$E(\pi_{ij}) = n_{ij}/n_i$$

uses observed proportions of fish size-classes in each mesh size as expected values for mesh selectivity s_{ij}, where π_{ij} = proportion of fish size j in mesh size i, n_{ij}/n_i, n_{ij} = catches of fish of size-class j in mesh i, and n_i = total catch of fish in mesh i, $\Sigma_j n_{ij}$. The population size–frequency, N_j, can be estimated by dividing the composite catch for each size interval, n_j, by the relative selectivity of that interval, S_{ij}, where

$$n_j = \Sigma_i n_{ij}$$

$$S_{ij} = \Sigma_i s_{ij} / \max_j(\Sigma_i s_{ij})$$

Estimation of selectivity

We estimated selectivity of each mesh size for fish size-classes from

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Table 1. Numbers of wedged and entangled lake trout by size interval in five mesh sizes of gill nets set during spring 1973 in Gull Island Refuge, Lake Superior.

Total length (mm)	Mesh size (mm)					
	102	114	127	140	152	Total
438	6	1	0	0	0	7
463	6	3	0	0	1	10
488	18	5	3	0	0	26
513	10	16	7	1	0	34
538	17	18	9	5	1	50
563	8	14	24	10	4	60
588	11	20	30	22	10	93
613	20	15	16	23	9	83
638	11	20	20	29	17	97
663	15	19	25	26	23	108
688	7	22	21	14	25	89
713	10	19	11	15	21	76
738	5	16	13	10	11	55
763	1	6	11	2	4	24
788	0	1	3	5	0	9
813	1	1	3	1	3	9
838	0	1	1	1	2	5
863	0	0	0	1	3	4
Total	146	197	197	165	134	839

Table 2. Numbers of wedged lake trout by size interval in five mesh sizes of gillnets set during spring 1973 in Gull Island Refuge, Lake Superior.

Total length (mm)	Mesh size (mm)					
	102	114	127	140	152	Total
438	6	0	0	0	0	6
463	5	3	0	0	0	8
488	16	4	1	0	0	21
513	9	14	6	1	0	30
538	9	17	6	5	0	37
563	6	10	20	7	4	47
588	6	14	23	20	9	72
613	6	6	11	20	8	51
638	7	10	13	23	14	67
663	5	7	13	15	21	61
688	0	7	11	8	23	49
713	1	7	2	8	16	34
738	1	5	4	4	9	23
763	0	1	1	1	1	4
788	0	0	0	1	0	1
813	1	0	0	0	2	3
838	0	0	0	1	0	1
863	0	0	0	0	3	3
Total	78	105	111	114	110	518

438 through 863 mm (25-mm intervals) by (1) computing the mean, standard deviation, and skewness of the sizes of fish caught in each mesh, (2) fitting linear relationships across mesh sizes for each moment of the fish size distribution, and (3) using the linear parameter estimates as seeds for the iterative least-squares search for parameters of the nonlinear model. We fit the model using SAS (SAS Institute Inc. 1989) and Systat (Systat Inc. 1992) procedures for nonlinear estimation, using the results from both derivative-dependent (Gauss–Newton and quasi-Newton, respectively) and derivative-free (DUD and SIMPLEX, respectively) algorithms as seeds for further searches (Seber and Wild 1989). We assumed that a global minimum sum of squares had been reached when all search methods converged on the same set of parameters for the nonlinear model.

Estimation of population size–frequencies

We estimated the relative abundances of the size-classes in the population by, first, estimating the total selectivity of the gear by summing the selectivity of the five mesh sizes for each fish size-class. Next, we estimated the relative selectivity of the gear for each fish size-class by dividing the total selectivity of the gear by the maximum total selectivity observed for any fish size-class. Last, we estimated the size–frequency of the population by dividing the observed catches by the relative selectivity of the gear for each fish size-class.

We used a bootstrap procedure to estimate 95% confidence intervals for the relative selectivity of the gear and for the estimated relative abundances of the various size-classes in the population (Efron 1979). We used the parameters, estimates, and residuals from the nonlinear iterative least-squares procedure outlined above as inputs for 1000 iterations of a bootstrap. In each iteration of the bootstrap, each predicted selectivity value was assigned a randomly selected residual (with replacement) from the set of residuals obtained in the nominal nonlinear iterative least-squares solution. The mean, 5% quantile, and 95% quantile from 1000 trials of the bootstrap procedure were used as estimates of the mean and its 95% confidence interval.

Bias in estimates of total annual survival

We constructed age-class distributions for estimating the bias in total annual mortality of lake trout caused by gillnet selectivity. The age–length key for lake trout caught in Wisconsin waters of Lake Superior in 1973 (Appendix) was used to construct age-class distributions from three size-class distributions of lake trout (Ricker 1975): (1) fish caught in 114-mm stretch measure nets, the standard assessment gear for indexing lake trout abundance and mortality in Lake Superior (Hansen et al. 1995), (2) fish caught in the five-mesh graded series from 102 through 152-mm stretch measure, and (3) the estimated population size–frequency for lake trout from the nonlinear least-squares estimation described above.

Age-class distributions were used to estimate rates of total instantaneous mortality (Z) from the descending limb of the catch curves (Ricker 1975). We used fish 9–11 years of age for estimating total instantaneous mortality because these three age-classes were represented in all three age-class distributions and were therefore fully vulnerable to the gear used to construct the age-class distributions. Estimates of total instantaneous mortality were then used to estimate annual survival rates (S), which were used to determine bias of the survival rates derived from catches in 114- and in 102- to 152-mm-mesh nets.

Results

Gillnet catches

Of the 839 lake trout caught in the five meshes, 62% (518 fish) were wedged and 38% (321 fish) were entangled (Tables 1 and 2). Both girth at the eye and girth at the dorsal fin were linearly related to lake trout total length (Fig. 1, top panel). Most entangled lake trout (98%; 316 of 321 fish) were larger than the upper 95% confidence interval of the maximum girth relationship and smaller than the lower 95% confidence interval of the eye girth relationship and could therefore have been wedged in the same mesh (Fig. 1, bottom panel). In contrast, only 2% (five fish) of the fish caught could not have been wedged in

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Fig. 1. Maximum girth (MG, upper line) and girth at the eye (EG, lower line) versus total length of lake trout caught in gill nets (top panel) and mesh opening perimeter versus total length of lake trout entangled in gill nets (bottom panel). All fish were caught in spring 1973 at Gull Island Refuge, Lake Superior. Parallel lines display means and 95% confidence intervals of individual observations and are plotted for comparison on the lower panel.

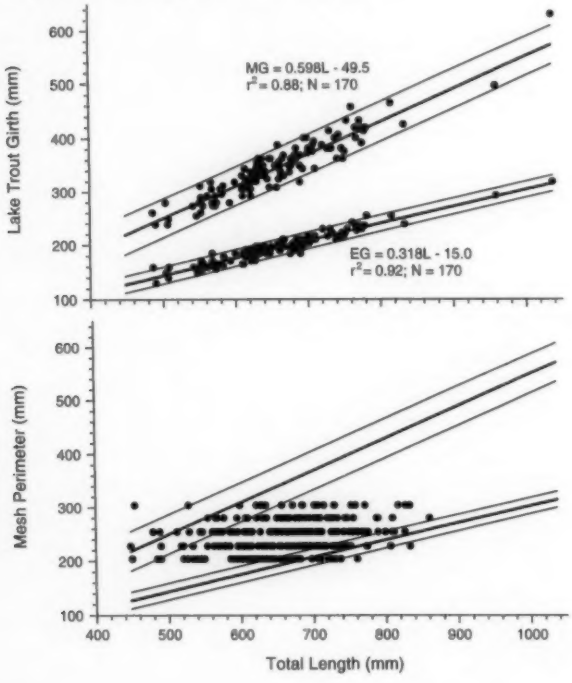
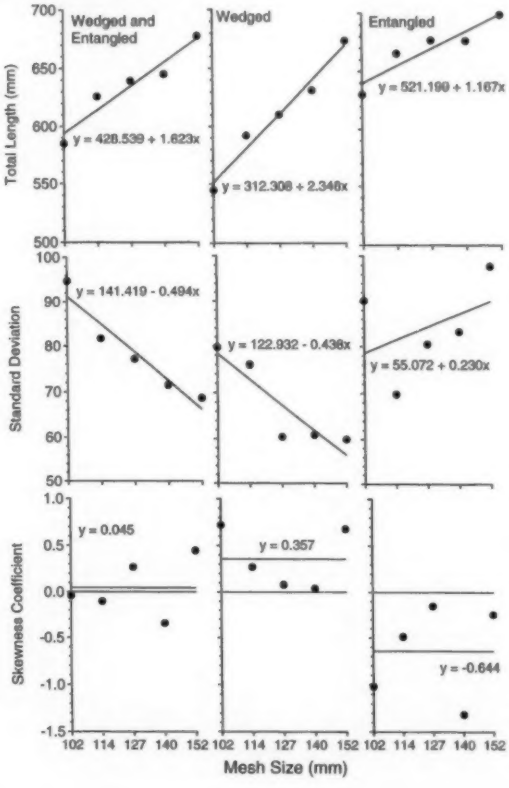


Fig. 2. Changes in the mean, standard deviation, and skewness of lake trout total length caught in gillnet mesh sizes of 102- to 152-mm stretch measure.



that mesh because they were either too small (two fish) or too large (three fish).

Mesh selectivity

Mean lengths of lake trout wedged or entangled in gill nets increased with mesh size, standard deviations decreased, and skewness varied without trend (Fig. 2, left panels). We fit a five-parameter model to the data on relative catches of lake trout wedged or entangled in the five meshes. The model had terms for a linear increase in mean length across meshes (slope, μ_1, and intercept, μ_0), a linear decrease in standard deviation of length across meshes (slope, σ_1, and intercept, σ_0), and no change in skewness of length across meshes (mean, q_0). The model explained 88% of variation in the proportions of fish of various sizes caught in the five mesh sizes (Table 3).

Mean lengths of lake trout wedged in gill nets increased with mesh size, standard deviations decreased, and skewness varied without trend (Fig. 2, center panels). We fit a five-parameter gillnet selection model to the data on relative catches of lake trout wedged in the five meshes. This model also had terms for a linear increase in mean length across meshes (slope, μ_1, and intercept, μ_0), a linear decrease in standard deviation of length across meshes (slope, σ_1, and intercept, σ_0), and no change in skewness of length across meshes (mean, q_0). The model explained 81% of variation in the proportions of fish of various sizes caught in the five mesh sizes (Table 3).

Mean lengths of lake trout entangled in gill nets increased with mesh size, standard deviations increased, and skewness varied without trend (Fig. 2, right panels). We again fit a five-parameter gillnet selection model to the data on relative catches of lake trout entangled in the five meshes. This model also had terms for a linear increase in mean length across meshes (slope, μ_1, and intercept, μ_0), a linear decrease in standard deviation of length across meshes (slope, σ_1, and intercept, σ_0), and no change in skewness of length across meshes (mean, q_0). The model explained 82% of variation in the proportions of fish of various sizes caught in the five mesh sizes (Table 3).

Selectivity of the five meshes for length-classes of wedged and entangled lake trout was skewed toward larger fish (Fig. 3). Peak selectivity increased from 588 to 663 mm for wedged and entangled lake trout as mesh size increased from 102 to 152 mm. Peak selectivity matched peak observed catches for each of the five meshes, although the shape of the selectivity curves for the three small meshes better matched dispersion of the observed catches than for the two large meshes. The five mesh selectivity curves appeared to have similar sizes and shapes, and therefore satisfied Baranov's geometrical similarity assumption for such analyses (as cited in Hamley 1975).

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Table 3. Parameter estimates, asymptotic confidence intervals, and model r^2 for wedged and entangled lake trout in five mesh sizes of gill nets set during spring 1973 in Gull Island Refuge, Lake Superior.

	Coefficient		Asymptotic 95% confidence interval	
Parameter	Initial	Final	Lower	Upper
	Wedged and entangled			
μ_0	428.539	242.376	149.233	335.519
μ_1	1.623	1.780	1.052	2.508
σ_0	141.419	232.689	137.662	327.716
σ_1	–0.494	–0.173	–0.915	0.569
q_0	0.045	–0.086	–0.096	–0.076
Model r^2	0.884			
	Wedged only			
μ_0	312.308	544.869	392.774	696.964
μ_1	2.346	1.894	0.714	3.074
σ_0	122.932	306.753	101.430	512.077
σ_1	–0.438	–0.591	–2.143	0.960
q_0	0.357	0.085	0.068	0.101
Model r^2	0.812			
	Entangled only			
μ_0	521.199	413.100	275.510	550.690
μ_1	1.167	0.594	–0.529	1.717
σ_0	55.072	156.988	–16.215	330.191
σ_1	0.230	0.632	–0.758	2.022
q_0	–0.644	–0.078	–0.091	–0.065
Model r^2	0.825			

Selectivity of the five meshes for wedged lake trout was skewed toward smaller fish whereas that for entangled lake trout was skewed toward larger fish (Fig. 4). As mesh size increased from 102 to 152 mm, peak selectivity for wedged lake trout increased from 563 to 663 mm, but for entangled lake trout increased from 613 to 688 mm. Peak selectivities of the five meshes rarely matched the peak observed catches for either wedged or entangled lake trout. The lack of overall model fit, both for wedged and for entangled lake trout, appeared to be caused by a change in the shape and size of the underlying selectivity of the gill nets, a violation of the geometrical similarity assumption.

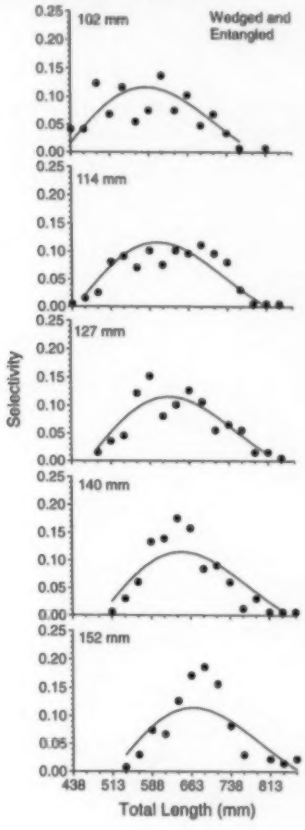
Relative selectivity

Relative selectivity peaked for lake trout 638 mm long that were wedged and entangled in the five gillnet meshes (Fig. 5). We did not estimate relative selectivity for separate catches of wedged and entangled lake trout because their mesh selectivity was poorly estimated. Relative selectivity was estimated with similar precision for all lengths of wedged and entangled lake trout, as evident by the relatively similar confidence intervals for the estimates at each length-class. Relative selectivity for wedged and entangled lake trout increased relatively smoothly from lake trout 438 mm long to the peak and decreased relatively smoothly from the peak to fish 863 mm long.

Population size–frequency

The size–frequency of the lake trout population that was estimated to have been caught in five gillnet mesh sizes was relatively broad and flat, compared with the size–frequency of the sample that was observed to have been wedged or entangled in the nets (Fig. 6). Relative numbers of lake trout 488–788 mm total length were estimated with greater precision than either smaller or larger size-classes, as seen from the relative widths of the confidence intervals around the estimates. Numbers of lake trout estimated in each length-class were greater than the observed catches for most length-classes, which shows how highly selective gill nets are for lake trout.

Fig. 3. Observed (points) and estimated (lines) mesh selectivity of lake trout wedged and entangled, combined, in gill nets in spring 1973, Gull Island Refuge, Lake Superior.



Bias in estimated annual survival

Age-class distributions of lake trout wedged and entangled in 114-mm single-mesh gill nets and in 102- to 152-mm graded-mesh gill nets were not greatly different from one another, but were each different from that of the estimated population (Fig. 7). Instantaneous total annual mortality (Z) of lake trout aged 9–11 years was 1.06 for catches in 114-mm nets and 1.03 for catches in 102- to 152-mm nets, but was only 0.57 for the estimated population. Accordingly, annual survival (S) of lake trout aged 9–11 years was 35% for catches in 114-mm nets and 36% for catches in 102- to 152-mm nets, but was 57% for the estimated population that accounted for gillnet selectivity.

Discussion

Indirect estimation of gillnet selectivity requires that the

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Fig. 4. Observed (points) and estimated (lines) mesh selectivity of lake trout wedged and entangled, separate, in gillnets in spring 1973, Gull Island Refuge, Lake Superior.

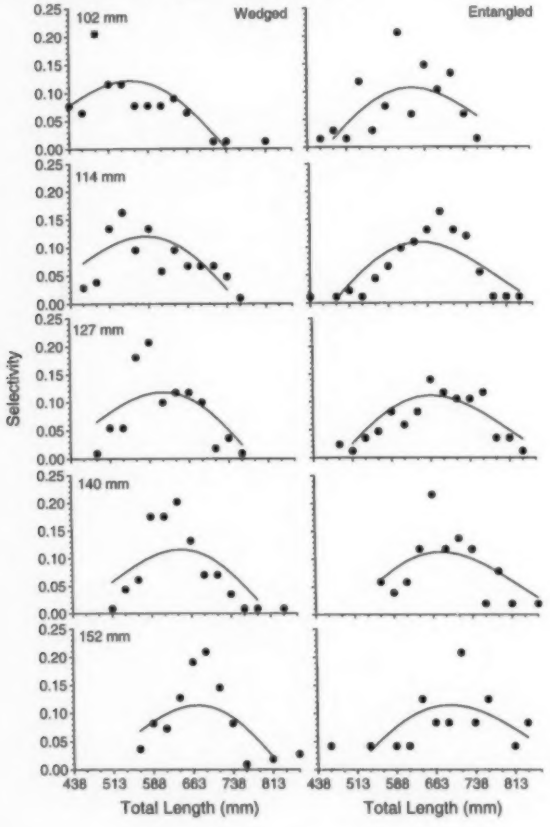
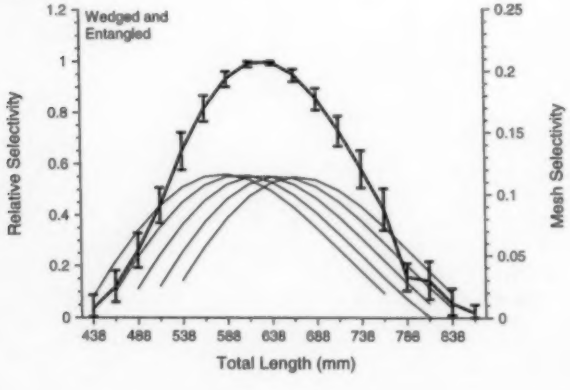


Fig. 5. Estimated mesh selectivity (from Fig. 3) and relative selectivity (mean ± 95% confidence intervals) of lake trout in five gillnet mesh sizes in spring 1973, Gull Island Refuge, Lake Superior.



selectivity curves for all meshes have the same size and shape (Baranov's geometrical similarity hypothesis; Hamley 1975). We found that the selectivity curves for wedged and entangled

Fig. 6. Observed catches (bars) and estimated population size–frequencies (mean ± 95% confidence intervals) of lake trout in five gillnet mesh sizes in spring 1973, Gull Island Refuge, Lake Superior.

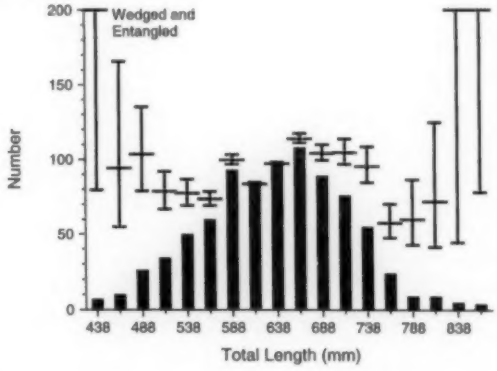
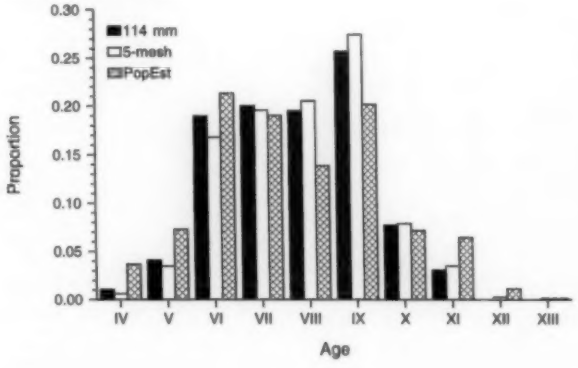


Fig. 7. Age–frequency distributions for lake trout caught in Wisconsin waters of Lake Superior in 114-mm single-mesh gill nets and 102- to 152-mm graded-mesh gill nets and from the estimated population.



lake trout in five meshes of gill nets were similar in size and shape, but not when wedged and entangled fish were modeled separately. Our conclusion is based on the observation that peak values and overall distributions of mesh selectivity matched observed catch frequencies for wedged and entangled lake trout, but not for separate catches of either wedged or entangled lake trout. The relatively poorer fit of the estimated selectivity of our largest two meshes to observed catches was likely caused by the rarity of lake trout larger than 800 mm total length in Gull Island Refuge in 1973. Only 5% of spawning-aged lake trout in Gull Island Refuge were older than age 10 in 1973 (Swanson and Swedberg 1980), the age at which most fish would have been larger than 800 mm total length. Because of the rarity of large lake trout in the Gull Island Refuge population, gillnet selectivity could not be accurately estimated from pairs of different meshes (e.g., Holt's method; Hamley 1975), rather than from the set of five meshes (Helser et al. 1991, 1994; Millar 1992, 1995). For example, selectivity estimated from our two largest meshes would imply a different shape of the selection curve (more peaked) than from all five meshes (more broadly domed). Contemporary

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catches in the two largest meshes are likely closer to our estimated population size–frequency because the proportion of large fish in the population in Gull Island Refuge has increased since 1973 (Schram et al. 1995).

A second assumption of indirect estimation of gillnet selectivity is that probabilities of fish encountering the net are equal for all sizes of fish (Helser et al. 1994). This assumption may be violated if fish of certain sizes are more likely to encounter the net than fish of other sizes (Hamley 1975). Rudstam et al. (1984) argued that this assumption is not true for fish that spend all or most of their time swimming, such as coregonines, scombrids, and carangids, and corrected their estimates of gillnet selectivity for cisco (*Coregonus artedi*) by assuming that encounter probability increased with fish length. However, Borgstrøm and Plahte (1992) contended that this approach is inappropriate for salmonines like brown trout (*Salmo trutta*) that make short excursions to catch prey fish. Tagging studies of lake trout in the Apostle Island region of Lake Superior have shown that few fish stray more than 80 km from their point of capture (Eschmeyer et al. 1953; Buettner 1961; Rahrer 1968). Of the few lake trout that strayed greater distances, Eschmeyer et al. (1953) showed that the incidence of moving more than 80 km was greater as fish increased from 225 to 500 mm total length, but there was no apparent increase in distance moved for fish larger than 500 mm total length. Since 95% of the lake trout caught in our nets exceeded 500 mm total length, we assumed that corrections of gillnet selectivity to account for size-based encounter probability were unwarranted.

A third assumption for indirect estimation of gillnet selectivity is that probabilities of fish being retained in the net are affected only by mesh size (Helser et al. 1994). This assumption may be violated if fish of a certain size tend to move along a portion of the net of certain mesh size into another portion of the net of another mesh size (Regier and Robson 1966). We minimized the likelihood of this bias by leaving gaps between the panels of different mesh sizes and by lifting the net after one night of fishing whenever possible (Regier and Robson 1966). Our one net that soaked four nights before it was lifted did not appear to be affected by saturation, as the total catch was similar to that obtained from summing one night's catch at the previous four nights' catch rate and three nights' catches at the succeeding two nights' catch rate.

The model we used to estimate gillnet selectivity was based on an assumed skew-normal distribution because lake trout may become tangled by their teeth and maxillaries, which generally causes selectivity curves to be broadly domed and skewed to the right (Hamley 1975), as we found in this study. In comparison, the selection curve for lake whitefish (*Coregonus clupeaformis*) (Regier and Robson 1966), a salmonid species without a large mouth, is relatively normal whereas the selection curves for other salmonids with larger mouths, such as rainbow trout (*Oncorhynchus mykiss*) (Fujimori et al. 1992), sockeye salmon (*Oncorhynchus nerka*), chum salmon (*Oncorhynchus keta*), pink salmon (*Oncorhynchus gorbuscha*) (reviewed by Hamley 1975), Arctic char (*Salvelinus alpinus*), and brown trout (reviewed by Jensen 1986), are more skewed.

Our estimates of gillnet selectivity should prove valuable in managing gillnet fisheries for lake trout in Lake Superior. Selectivity can be incorporated into Ricker equilibrium models in the same manner as was done by Madenjian and Ryan (1995) to arrive at recommended allowable harvests for gillnet fisheries for lake trout in Lake Superior. Estimated gillnet selectivity can also be used to understand and adjust for bias in estimates of lake trout mortality from lake trout catches in single-mesh and graded-mesh assessment nets. We found that estimated annual survival was biased low by more than 20%, whether estimated from catches in 114-mm single-mesh nets or from catches in 102- to 152-mm graded-mesh nets. Bias of this magnitude in estimated annual survival should be taken into account in setting limits for total allowable harvest of lake trout in Lake Superior.

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Appendix. Proportion of lake trout per age-class within various length-classes caught in spring gillnet assessment fisheries in Wisconsin waters of Lake Superior in 1973.

Total length	Age (years)									
(mm)	4	5	6	7	8	9	10	11	12	13
438	0.18	0.22	0.37	0.18	0.04	0.00	0.00	0.00	0.00	0.00
463	0.06	0.22	0.45	0.14	0.03	0.10	0.00	0.00	0.00	0.00
488	0.02	0.06	0.34	0.56	0.01	0.00	0.00	0.00	0.00	0.00
513	0.04	0.12	0.52	0.29	0.04	0.00	0.00	0.00	0.00	0.00
538	0.03	0.15	0.35	0.40	0.07	0.00	0.00	0.00	0.00	0.00
563	0.00	0.02	0.60	0.30	0.08	0.01	0.00	0.00	0.00	0.00
588	0.00	0.12	0.22	0.35	0.22	0.09	0.00	0.00	0.00	0.00
613	0.00	0.00	0.21	0.29	0.36	0.14	0.00	0.00	0.00	0.00
638	0.00	0.00	0.09	0.23	0.37	0.24	0.08	0.00	0.00	0.00
663	0.00	0.00	0.01	0.11	0.14	0.61	0.12	0.00	0.00	0.00
688	0.00	0.00	0.01	0.04	0.29	0.51	0.15	0.00	0.00	0.00
713	0.00	0.00	0.05	0.01	0.23	0.50	0.12	0.09	0.00	0.00
738	0.00	0.00	0.00	0.02	0.24	0.37	0.21	0.15	0.00	0.02
763	0.00	0.00	0.00	0.04	0.07	0.39	0.29	0.21	0.00	0.00
788	0.00	0.00	0.00	0.00	0.00	0.38	0.13	0.50	0.00	0.00
813	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.50	0.25	0.00

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Environmental controls of phytoplankton bloom dynamics in the Neuse River Estuary, North Carolina, U.S.A.

James L. Pinckney, David F. Millie, Bryan T. Vinyard, and Hans W. Paerl

Abstract: Spatial and temporal patterns of phytoplankton growth, biomass, primary productivity, and community composition were linked to environmental variables to identify key factors promoting algal blooms in the moderately eutrophic Neuse Estuary. Diagnostic photopigments (carotenoids), used to examine phytoplankton community structure, explained 83.3% (n = 567) of the variation in phytoplankton biomass (chlorophyll *a*). Principal factor analysis identified hydrological forcing variables (salinity, K_d, NO_x, and NH_4^+) that explained 71% of total variability associated with phytoplankton biomass. Phytoplankton carbon-specific growth rates (μ_{Chl}), measured using chlorophyll *a* ^{14}C-labeling, ranged from 0.01 to 0.83·day^{-1} (0.27 ± 0.22, mean ± SD) and decreased downstream. A regression model ($R^2 = 0.41$, $p < 0.01$), derived from 25 months of biweekly sampling of 13 environmental variables, was used to predict μ_{Chl} and construct spatiotemporal contour plots of phytoplankton growth. Enhanced growth (bloom initiation) occurs in the upper riverine portion of the estuary, biomass accumulates (proliferation and full bloom) in the central portion, and productivity (but not μ_{Chl}), overall, tracks phytoplankton abundance. Management strategies for stemming eutrophication should target processes and conditions associated with bloom initiation in the riverine rather than the estuarine portion of the system.

Résumé : Nous avons établi des liens entre les schémas spatiaux et temporels de la croissance du phytoplancton, de sa biomasse, de la productivité primaire et de la composition de la communauté avec des variables environnementales en vue de repérer les facteurs clés qui favorisent les efflorescences algales dans l'estuaire modérément eutrophe de la Neuse. Les photopigments diagnostiques (caroténoïdes), qui ont servi à examiner la structure de la communauté de phytoplancton, expliquaient 83,3 % (n = 567) de la variation de la biomasse phytoplanctonique (chlorophylle *a*). L'analyse des facteurs principaux a mis en évidence des variables de forçage hydrologique (salinité, K_d, NO_x et NH_4^+) qui expliquaient 71 % de la variabilité totale observée dans la biomasse phytoplanctonique. Les taux de croissance du phytoplancton spécifiques au carbone (μ_{Chl}), mesurés par marquage au ^{14}C de la chlorophylle *a*, allaient de 0,01 à 0,83·jour^{-1} (0,27 ± 0,22, moyenne ± ET), et diminuaient vers l'aval. Un modèle de régression ($R^2 = 0,41$, $p < 0,01$), calculé d'après 25 mois d'échantillonnage bi hebdomadaire de 13 variables environnementales, a servi à prédire μ_{Chl} et à construire des isogrammes spatiotemporels de la croissance du phytoplancton. Une poussée de croissance (début de l'efflorescence) se produit dans la portion fluviale supérieure de l'estuaire, la biomasse s'accumule (prolifération et maximum de l'efflorescence) dans la portion centrale, et la productivité (mais non μ_{Chl}) suit dans l'ensemble l'abondance du phytoplancton. Les stratégies de gestion visant à enrayer l'eutrophisation doivent cibler les processus et les conditions qui président au début de l'efflorescence, dans la portion fluviale plutôt que la portion estuarienne du système.
[Traduit par la Rédaction]

Introduction

Phytoplankton blooms are a result of rapid microalgal growth, which produces high biomass and densities that persist for days to weeks. Nuisance phytoplankton blooms are increasing in frequency and extent in estuarine and coastal waters worldwide (Paerl 1988; Hallegraeff 1993). Such blooms often yield harmful short-term (toxicity, water taste or odor problems, hypoxia and anoxia) and long-term (loss of fisheries and recreational resources, biogeochemical and trophic alterations) consequences for water-quality and resource use. Although negative water-quality impacts are well documented and of considerable concern, the exact conditions and mechanisms promoting bloom initiation, proliferation, and persistence are poorly understood (Margalef 1978; Thomas and Gibson 1990; Glibert et al. 1995; Kononen et al. 1996). Because the time and location of phytoplankton blooms are difficult to predict in stochastic estuarine habitats, researchers often assess the sequence of events leading to altered productivity and community composition. Most often, such changes are related to the exposure history of phytoplankton to physical and chemical regimes. However, in rivers, wetlands, and estuaries, phytoplankton biomass and composition at a particular location often reflect growth incurred upstream. As such, the product of physiological rate processes (e.g., phytoplankton standing stock) may be separated spatially from the region where the

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processes originated or achieved maximum rates. Thus, the causes and effects of algal blooms may be separate in space and time.

Estuarine phytoplankton communities are composed of diverse taxa, each having distinct physiological characteristics. In addition, these assemblages exhibit patchy distributions over variable spatial and temporal scales. The dynamic interplay of environmental conditions coupled with the unique physiological responses of individual taxa enables bloom-forming species to outcompete other taxa and establish nearly monotypic biomass accumulations. Further, the distinct environmental gradients and ecotones regulating phytoplankton dynamics are often spatially explicit. For example, river discharge, frontal zones, vertical stratification, and mixing rates influence phytoplankton distributions and may enhance growth and productivity (Dustan and Pinckney 1989; Pinckney and Dustan 1990; Cloern 1991; Franks 1992; Klarer and Millie 1994; Kononen et al. 1996). Consequently, temporal patterns of phytoplankton assemblages and physiological characteristics can provide insights into the factors and timing of events that mediate algal bloom potentials.

The environmental factors responsible for promoting and establishing phytoplankton blooms need to be characterized within relevant temporal and spatial scales before effective management strategies that target regions and periods of maximum bloom potentials in an estuary can be developed. To further link phytoplankton community dynamics and physiological processes at the ecosystem level, we examined the spatiotemporal characteristics of physical and chemical factors affecting phytoplankton growth, productivity, and resultant community compositional dynamics in the moderately eutrophic Neuse River Estuary, North Carolina, U.S.A.

Study site

The Neuse watershed drains 16 000 km^2 of North Carolina's most productive and rapidly expanding urban, industrial, and agricultural regions. The river flows through 300 km of agriculturally dominated Coastal Plain before emptying into the Neuse Estuary (35°00′N, 76°45′W) and Pamlico Sound (Fig. 1). The river–estuarine system is shallow (<5 m) and salinity varies with river discharge, meteorological conditions (precipitation and wind), and saltwater influx from Pamlico Sound. Tidal influence is minimal and circulation is primarily gravitational (Robbins and Bales 1995). Water column density (salinity) stratification occurs during high river discharge or long periods of calm weather (i.e., no vertical mixing) (Paerl et al. 1995).

Diverse and changing land-use activities in the watershed generate various nonpoint-source nutrient inputs, including fertilizer runoff and soil erosion. Agricultural production and fertilizer use have expanded markedly in the past 40 years (Stanley 1988; Copeland and Gray 1989). In addition, poultry, swine, and cattle farms contribute to nutrient loading. Because of chronic and excessive nitrogen and phosphorus loading and resulting increases in the frequency and areal coverage of nuisance algal blooms, the Neuse River has recently received nutrient-sensitive designation by the state and listing as one of the 20 most threatened rivers in the United States (American Rivers, Washington, D.C. 1996). Phytoplankton primary production in both the freshwater and estuarine portions of the river is controlled primarily by nitrogen (N) availability (i.e., N limited) throughout much of the year (Paerl 1987; Rudek et al. 1991; Boyer et al. 1994). During the past two decades, alarming symptoms of eutrophication, including nuisance phytoplankton blooms, bottom water hypoxia and anoxia, fish kills, and altered food web structure have plagued water quality in the lower Neuse River (Paerl 1983, 1987; Christian et al. 1986).

Phytoplankton growth in the Neuse is regulated, in part, by the degree of and interplay among environmental factors. For example, high spring rainfall introduces nonpoint-source nutrients through direct deposition and stormwater runoff into the upper reaches of the estuary. In the wider segments of the river, downstream currents can slow to virtually undetectable rates (water residence times in the lower reaches may exceed 2 months; Christian et al. 1991; Robbins and Bales 1995). Nutrients discharged during spring freshets are retained in the broad, lower reaches of the estuary as it vertically stratifies and stagnates in a "lakelike" fashion during the calm and relatively dry summer and fall months (Paerl 1987; Showers et al. 1990). Such conditions have promoted the periodic development and proliferation of nuisance cyanobacterial (*Microcystis aeruginosa*) blooms within the freshwater portions of the river (Paerl 1983, 1987; Christian et al. 1986; Lung and Paerl 1988). Extensive winter–spring blooms of dinoflagellates (*Heterocapsa triquetra*, *Gymnodinium minutum*) and cryptomonads (*Cryptomonas* sp.) are common and increasing in intensity and duration within the oligo- and meso-haline segments of the estuary (Mallin 1994).

Materials and methods

Sampling

Water samples and ambient physical and chemical data were examined at six sites (ranging from freshwater to mesohaline) in the Neuse River Estuary at biweekly intervals from January 1994 through February 1996 (Fig. 1). Vertical profiles of conductivity, salinity, temperature, dissolved O_2, and pH throughout the water column were obtained at each site with a Hydrolab H20 probe coupled to a Surveyor 3 datalogger. Diffuse attenuation coefficients (K_d) were calculated based on vertical profiles of spherical irradiance (photosynthetically active radiation) measured with LI-COR 4π spherical sensor (192S). Water samples were collected near the water surface (0.5 m depth) and at depth (0.5 m from the bottom), transferred to acid-cleaned 20-L carboys, and kept cool and shaded during transport to the laboratory. Subsamples for nutrient, photopigment, and primary productivity analyses were removed from bulk water samples within 3 h of collection. Measurements of carbon-specific phytoplankton growth rates were obtained for samples at selected sites and dates (see below).

Nutrient analyses

Water samples were filtered through precombusted (500°C, 16 h) Whatman GF/F glass-fiber filters before chemical analyses. Nitrite + nitrate ($NO_2^- + NO_3^-$; hereafter NO_x) concentrations were determined using a spongy cadmium shaking technique (Jones 1984). Ammonium (NH_4^+) was measured using the phenol–hypochlorite method (Solorzano 1969). Dissolved inorganic phosphate (PO_4^{3-}) was quantified using the molybdate-blue method corrected for silicon and arsenic interference (Strickland and Parsons 1972).

Phytoplankton photopigments

Phytoplankton community composition was characterized using high-performance liquid chromatographic (HPLC) derived photopigment

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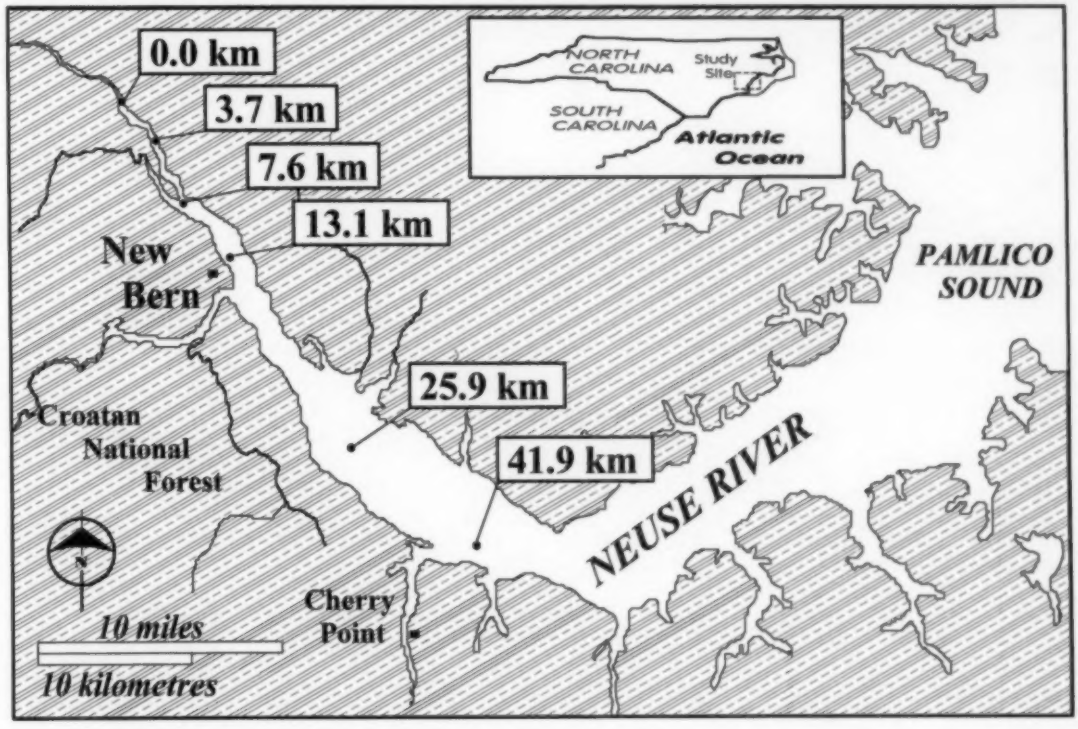
Fig. 1. Locations of sampling sites in the Neuse River. Values for each station represent distance downstream from most upstream site, 0 km.



profiles (Millie et al. 1993; Tester et al. 1995; Pinckney et al. 1996). Aliquots (0.3–1 L) of surface and bottom water were filtered under a gentle vacuum (<50 kPa) onto GF/F filters, immediately frozen, and stored at 20°C. Frozen filters were placed in 100% acetone (2 mL), sonicated, and extracted at 20°C for 12 h. Filtered extracts (200 µL) were injected into a Spectra-Physics HPLC equipped with a single monomeric (Rainin Microsorb-MV, 0.46 × 10 cm, 3 mm) and two polymeric (Vydac 201TP, 0.46 × 25 cm, 5 mm) reverse-phase C_{18} columns in series. A nonlinear binary gradient, adapted from Van Heukelem et al. (1992), was used for pigment separations (for details, see Pinckney et al. 1996). A Shimadzu Model SPD-M10av photodiode array detector (PDA) was used to obtain absorption spectra and chromatograms (440 nm). Pigment peaks were identified by comparison of retention times and absorption spectra to those of authentic crystalline standards, including chlorophyll *a* (Chl *a*), chorophyll *b* (Chl *b*), β-carotene (Sigma Chemical Co.), fucoxanthin, and zeaxanthin (Hoffman-LaRoche & Co.). All other pigments were identified by comparison with pigments extracted from phytoplankton cultures (Wright et al. 1991) and quantified using the appropriate extinction coefficients (Mantoura and Llewellyn 1983; Rowan 1989).

Phytoplankton productivity

Incubations were conducted in an outdoor, flow-through seawater pond at the Institute of Marine Sciences. A field light simulator (described in Mallin and Paerl 1992) was used to mimic the variable ambient light regime (5–100% surface irradiance) associated with vertical mixing. Surface water samples from each station were injected with $NaH^{14}CO_3$ (final concentration 185–260 kBq·mL^{-1}) and incubated in racks beneath the simulator. Triplicate light and single dark incubations of 3–4 h (centered around 12:00) were completed for each site. After incubation, phytoplankton were filtered onto GF/F filters, air dried, and fumed with concentrated HCl to remove unincorporated 14C. Filters were then placed in vials containing scintillation cocktail (Ecolume; ICN, Inc.) and the counts per min (CPM) enumerated with Beckman model LS5000TD liquid scintillation counter. CPMs were converted to disintegrations per min (DPMs) using quench curves constructed from a calibrated ^{14}C-toluene standard. Dissolved inorganic carbon in water samples was determined by infrared gas analysis (Beckman model 864 IRGA) (Paerl 1987).

Phytoplankton community growth rates

Carbon-specific growth rates of phytoplankton assemblages (μ_{CM}) were derived using the Chl *a* radiolabeling method coupled with HPLC-based photopigment analyses (Redalje 1993; Pinckney et al. 1996) and calculated using the model of Goericke and Welschmeyer (1993). Water samples for analyses were obtained for surface assemblages at specific sites (0, 13.1, 25.9, and 41.9 km, Fig. 1), on selected dates. These sites were chosen to cover the range of salinities (0–20‰) and phytoplankton representative of the study area (Mallin 1994; Paerl et al. 1995). Radiolabeling was accomplished by incubating phytoplankton in the presence of $NaH^{14}CO_3$ and measuring the specific activity of ^{14}C in Chl *a*. Briefly, radioisotope was added to 1-L water samples (in triplicate) to achieve an activity of 3330 kBq·L^{-1}. Phytoplankton samples then were incubated outdoors in the seawater pond under in situ irradiance and temperature. Incubations were conducted from dawn to dawn (24 h) to allow sufficient ^{14}C incorporation and turnover of precursor photopigment pools (Redalje and Laws 1981; Goericke and Welschmeyer 1993; Redalje 1993; Riemann et al. 1993). For measuring the ^{14}C-specific activity of Chl *a*, an in line flow scintillation counter (Packard Radiomatic 525*a*, 500-µL counting cell) was placed downstream from the PDA. Radioactivity was quantified after automatic, in-line mixing of a low-viscosity scintillation cocktail (Packard Ultima-Flo M) with HPLC eluant (3:1 mixing ratio). Radiodetector data were converted to DPMs after accounting for variable flow rates, counting cell volume, mixing rates, and counting efficiency (Pinckney et al. 1996).

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Table 1. Summary statistics for physical and chemical variables in surface waters at sampling sites during the study period.

Variable	Parameter	Location (km) 0.0	3.7	7.6	13.1	25.9	41.9
Temperature (°C)	Mean (SE)	18.4 (1.1)	18.6 (1.1)	18.5 (1.1)	18.9 (1.1)	18.7 (1.1)	18.7 (1.1)
	Median	19.8	20.0	20.5	20.0	20.2	20.0
	Range	3.3–30.5	3.4–31.1	3.0–30.4	3.6–31.0	3.3–30.9	3.8–30.3
pH	Mean (SE)	7.4 (0.1)	7.4 (0.1)	7.3 (0.1)	7.4 (0.1)	7.3 (0.1)	7.2 (0.1)
	Median	7.6	7.4	7.3	7.4	7.4	7.3
	Range	4.3–9.0	4.3–8.9	4.3–9.3	4.7–9.1	5.2–8.3	5.5–8.6
Salinity (‰)*	Mean (SE)	0.1 (0.0)	0.3 (0.1)	0.8 (0.2)	2.2 (0.4)	6.7 (0.7)	9.7 (0.8)
	Median	0.1	0.1	0.1	0.9	6.0	8.9
	Range	0.0–0.4	0.0–3.1	0.0–6.8	0.0–10.9	0.0–18.7	1.0–19.6
Conductivity (mmhos·cm^{-1})*	Mean (SE)	0.2 (0.2)	1.1 (0.6)	2.1 (0.7)	4.5 (0.9)	11.3 (1.3)	16.8 (1.2)
	Median	0.1	0.2	0.2	2.0	10.5	15.7
	Range	0.0–0.8	0.1–28.6	0.0–31.0	0.1–32.8	0.1–35.9	3.8–31.5
Attenuation coefficient (m^{-1})*	Mean (SE)	2.5 (0.1)	2.7 (0.1)	2.7 (0.1)	2.6 (0.1)	2.4 (0.1)	2.1 (0.1)
	Median	2.4	2.7	2.6	2.5	2.1	2.0
	Range	1.5–4.9	1.3–3.9	1.9–4.0	0.8–4.2	1.2–6.4	0.3–4.5
Nitrate +nitrite (μg N·L^{-1})*	Mean (SE)	527 (38)	483 (36)	440 (32)	337 (32)	108 (21)	38.9 (8.1)
	Median	529	455	417	302	32.9	13.3
	Range	70–1303	30–1327	47–1100	2–799	0–572	1–259
Ammonium (μg N·L^{-1})*	Mean (SE)	61.2 (4.8)	106.9 (7.7)	102.2 (7.6)	77.6 (7.3)	26.0 (2.9)	17.4 (1.5)
	Median	58.4	102.1	107.0	70.1	22.4	16.3
	Range	1.4–197.1	0.3–266.4	12.1–254.8	0.8–206.9	3.8–104.0	0.0–51.2
Phosphate (μg P·L^{-1})*	Mean (SE)	64.6 (4.6)	70.4 (5.6)	69.2 (5.0)	65.5 (5.5)	47.6 (6.7)	38.4 (6.0)
	Median	63.5	69.9	66.6	51.6	39.8	25.4
	Range	1.2–135.0	14.0–220.4	10.4–153.6	8.8–185.1	2.1–291.1	0.8–157.2
Dissolved O_2 (mg O_2·L^{-1})*	Mean (SE)	7.9 (0.3)	7.8 (0.3)	7.6 (0.3)	8.3 (0.3)	9.1 (0.2)	9.2 (0.3)
	Median	7.7	7.4	7.2	8.2	8.9	8.9
	Range	4.3–12.9	3.9–12.3	3.9–12.3	4.0–14.5	6.5–12.5	6.0–14.6

Note: The mean and SE represent all collections at each station ($n = 46$). An asterisk indicates variables that increased or decreased throughout the length of the estuary as determined by regression analysis ($n = 6$, $p < 0.01$).

Statistical analyses

Environmental (physical and chemical) variables were sorted and a mean, median, and range determined for each station ($n = 46$). Significant trends (increasing or decreasing in a downstream direction) were determined by regression analysis using the mean values for each station ($n = 6$). Multiple linear regression was used to assess the phylogenetic group-specific contribution to total Chl *a* (Gieskes and Kraay 1983; Tester et al. 1995). First, the HPLC-derived Chl *a* : diagnostic accessory pigment ratios were established for each phylogenetic group by the regression. Using the appropriate regression coefficients, the contribution of each group to the total Chl *a* biomass then was calculated and plotted. The similarity in trends between measured and predicted (from the regression equation) Chl *a* for surface and bottom assemblages was assessed by an analysis of covariance (Snedecor and Cochran 1980). Measured and predicted Chl *a* for assemblages in surface and bottom waters were plotted to identify specific instances of variability in Chl *a* : accessory pigment ratios. The correlation structure among biological, chemical, and physical variables was examined by a principal factor analysis (PFA) using "oblique" rotation of the factor structure, thereby allowing for correlation among common factors (Tabachnick and Fidell 1983). The purpose of this analysis was to identify chemical or physical factors at a particular site and (or) upstream sites that may be forcing Chl *a* biomass and (or) productivity. Variables for each site and date and variables from sampling sites and dates immediately preceding these sites and dates (hereafter referred to as lagged (L) variables) were analyzed. Missing data for a small number of sampling dates were estimated using an adaptation of the nearest-neighbor algorithm (Strutton et al 1996).

Phytoplankton growth rates were compared using a randomized, complete block (sampling date) analysis of variance (ANOVA; Snedecor and Cochran 1980). To satisfy the assumptions of ANOVA, values were transformed to natural logarithms (ln). Linear regression analyses (Snedecor and Cochran 1980) of ln-transformed μ_{Chl} and ln-transformed Chl *a*, productivity, and assimilation numbers were used to detect relationships within paired samples (i.e., samples collected and incubated on the same dates).

A stepwise (forward-selection) multiple regression model was used to relate μ_{Chl} to contemporaneous in situ environmental conditions. Pooled biweekly physical and chemical data were used in the model to calculate predicted μ_{Chl}, which then were plotted against measured μ_{Chl} and Chl *a*. Contour plots of predicted μ_{Chl}, Chl *a*, and productivity were constructed for comparisons of these variables through space and time. A kriging algorithm (linear variogram model; Surfer version 6.02 software) was used for interpolation between points for contours.

Results

Water-quality variables in the surface waters for each station were summarized to compare conditions along the transect (Table 1). Mean salinity, conductivity, and dissolved O_2 concentrations displayed increasing trends downstream, whereas diffuse attenuation coefficient values and NO_x, NH_4^+, and PO_4^{3-} concentrations decreased from the upper to the lower stations ($p < 0.01$). Mean temperature and pH were similar at all six stations.

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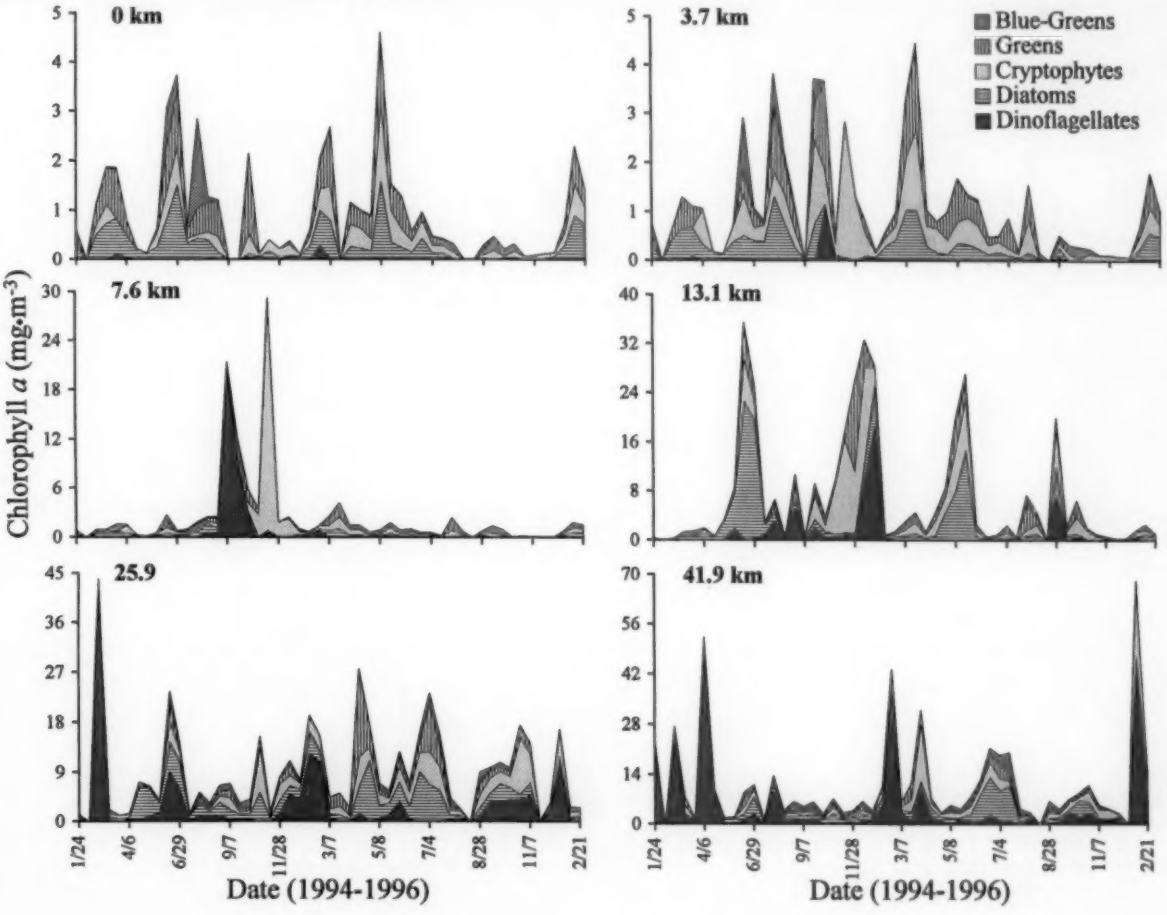
Fig. 2. Group-specific contribution to the total Chl *a* biomass, as determined from multiple linear regression of HPLC-derived Chl *a* : accessory pigment ratios (see text) for each sampling site.



Relationships between phytoplankton community composition and Chl *a* concentrations were evaluated using the pooled photopigment data. The phylogenetic, group-specific contribution to total Chl *a* was determined by a multiple linear regression equation establishing the Chl *a* : accessory pigment ratio (i.e., regression coefficients ± SE) for each group. An adequate predictor of HPLC-derived Chl *a* concentrations of assemblages in surface and bottom waters ($p < 0.01$) was

$$\begin{aligned}[\text{Chl } a] = {} & 0.85\pm0.03\ [\text{Peridinin}] \\ & + 1.02\pm0.08\ [\text{Fucoxanthin}] \\ & + 2.06\pm0.14\ [\text{Alloxanthin}] \\ & + 3.80\pm0.51\ [\text{Chl } b] \\ & + 0.27\pm0.09\ [\text{Zeaxanthin}] + 0.70\pm0.20\end{aligned}$$

where peridinin, fucoxanthin, alloxanthin, Chl *b*, and zeaxanthin were concentrations (mg·m^{-3}) of accessory pigments indicative of dinoflagellates, diatoms, cryptophytes, chlorophytes, and cyanophytes, respectively. The intercept value (0.70) approximates 0 and illustrates that the five pigment groups explain most of the variability associated with Chl *a* (cf. Gieskes and Kraay 1983). The diagnostic pigments used in the regression equation accounted for 83.3% ($n = 567$) of the variation in measured Chl *a* concentrations.

Phytoplankton biomass and composition were variable within and among sampling sites (Fig. 2). Sites in the upper reaches of the study area (0 and 3.7 km) were characterized by low Chl *a* concentrations (<5.4 mg Chl *a*·m^{-3}). Generally, the phytoplankton communities at these sites were mixed assemblages of diatoms, cryptophytes, and cyanophytes (Fig. 2). At the oligohaline middle stations (7.6 and 13.1 km), Chl *a* typically was slightly greater (3.0 to 8.5 mg·m^{-3}) than in the upper reaches. During blooms of diatoms, dinoflagellates, and (or) cryptophytes (Fig. 2), Chl *a* reached up to 53 mg·m^{-3}. In the mesohaline lower reaches of the estuary (25.9 and 41.9 km), the mean Chl *a* was 14 mg·m^{-3}. Frequent dinoflagellate, cryptophyte, and (to a lesser extent) chlorophyte blooms produced Chl *a* concentrations as high as 90 mg·m^{-3}. The trends in measured (HPLC-derived) and predicted (by the regression model) Chl *a* generally were similar at all sites. Although no difference in the overall fit of the regression existed between assemblages in surface and bottom waters ($p > 0.05$), the model more adequately accounted for variation in Chl *a* in

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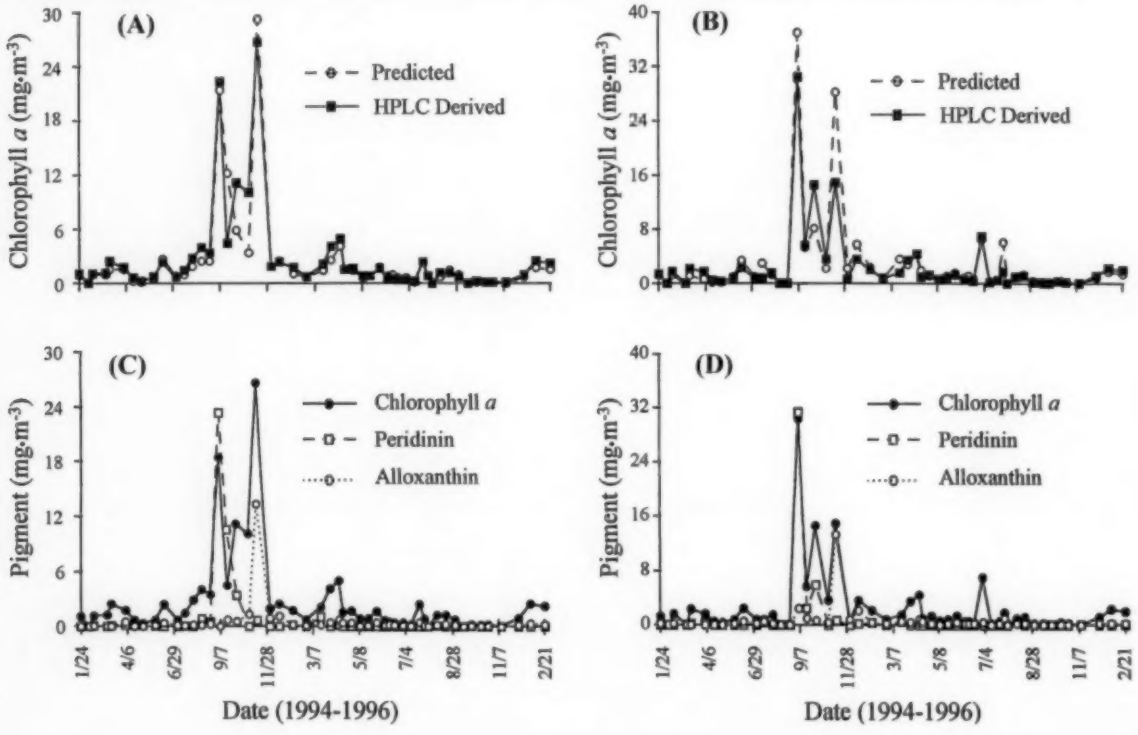
Fig. 3. Pigment concentrations for assemblages at sampling site, 7.6 km (refer to Fig. 1). (A and B) HPLC-derived and predicted (calculated from regression model, see Materials and methods section) Chl *a* concentrations for assemblages in surface and bottom waters, respectively. (C and D) HPLC-derived Chl *a*, peridinin, and alloxanthin concentrations for assemblages in surface and bottom waters, respectively.



surface waters (88.3%, $n = 288$) than in bottom waters (79.5%, $n = 279$). Differences between measured and predicted Chl *a* were most evident during specific bloom events, particularly those dominated by cryptophytes and dinoflagellates (e.g., Figs. 3A and 3B). Differences in the Chl *a* : alloxanthin (and to a lesser extent, Chl *a* : peridinin) ratio(s) between assemblages in surface and bottom waters also were evident during cryptophyte and dinoflagellate blooms (Figs. 3C and 3D).

Biomass-specific phytoplankton productivity (i.e., productivity divided by Chl *a* concentration or assimilation number) decreased from the upper to the lower stations ($p < 0.01$), ranging from 38.6 to 0.4 mg C·mg Chl $a^{-1}\cdot h^{-1}$. The greatest assimilation numbers (9.6 ± 1.0; mean ± SE) occurred in the middle reaches (7.6 and 13.1 km) of the estuary, whereas the lowest values (5.8 ± 0.5) were found in the lower reaches (25.9 and 41.9 km).

Principal factor analysis was used to examine the correlation structure among physical, chemical, and biological response variables. Originally, a variety of PFAs, each selecting for a distinct number of factors, were attempted on data sets both for the entire Neuse River and paired sampling locations (e.g., sites 0 and 3.7 km, 3.7 and 7.6 km, etc.). Depending upon the analysis, four to six factors explained most of the variability within the data (as determined from eigenvalues approximating 1.0). The remaining factors chosen by the PFA could not significantly improve on explaining variability and, therefore, were not considered. The PFA identified four factors explaining 71% of the total variability (Table 2). Factor 1 (variables indicative of river discharge) was the prominent descriptor (explaining 31%) of variability. Within the PFAs conducted on data for both the whole study region and for paired sites, salinity, K_d, conductivity, and NO_x concentrations always loaded together within a factor. However, temperature and PO_4^{3-} were always associated with phytoplankton assimilation numbers (see factor 2) for all sites. Within factors, NH_4^+ and K_d were positively correlated with one another and negatively correlated with salinity and conductivity (factor 1), whereas assimilation number, PO_4^{3-}, and temperature were positively correlated with one another and negatively correlated with dissolved O_2 (factor 2). The association of riverine discharge (factor 1) upon lagged phytoplankton assemblages (factor 4) was approximately twice as great ($r = 0.43$ vs. 0.24) as that upon assemblages (factor 3) at the time of sampling.

Phytoplankton community growth rate (μ_{Chl}) measurements were obtained for 41% of the sampling dates during the study. Sixty-three, triplicate incubations (encompassing 20 sampling dates for sites 0 and 41.9 km, 13 dates for site 13.1 km, and 10 dates for site 25.9 km) were conducted. Growth rates ranged from 0.01 to 0.83 day^{-1} (0.27 ± 0.22 (mean ± SD)) and were distinct among sampling sites ($p <$ 0.01). Greater values were observed at upstream sites (site 0 km: 0.33 ± 0.20; site 13.1 km: 0.43 ± 0.29) than downstream sites (site 25.9 km: 0.17 ± 0.12; site 41.9 km: 0.16 ± 0.14) (Fig. 4). Growth rates also were distinct among sampling dates

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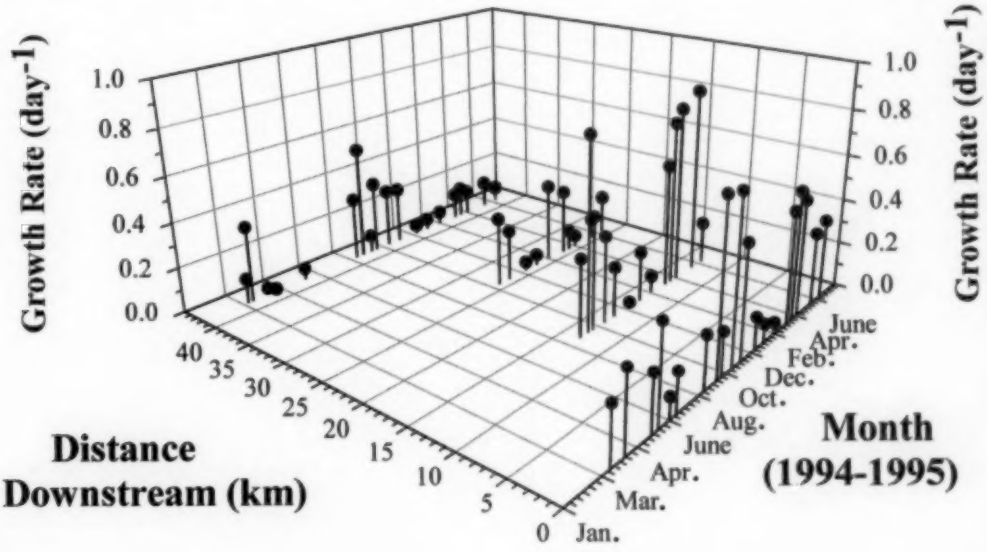
Table 2. Results of a principal factor analysis (using oblique rotation) examining the correlation structure among response variables for the Neuse River data set.

Factor 1 (0.31)		Factor 2 (0.50)		Factor 3 (0.62)		Factor 4 (0.72)	
Variable	Loading	Variable	Loading	Variable	Loading	Variable	Loading
Salinity	0.81	Temperature	0.86	Chl *a* (predicted)	0.94	Chl *a* (predicted) (L)	0.77
Salinity (L)	0.79	Temperature (L)	0.86	Chl *a* (measured)	0.88	Diatom Chl *a* (L)	0.76
Conductivity	0.78	$[PO_4^{3-}]$	0.66	Dinoflagellate Chl *a*	0.69	Chl *a* (measured) (L)	0.69
Conductivity (L)	0.78	$[PO_4^{3-}]$ (L)	0.57	Diatom Chl *a*	0.68	Chlorophyte Chl *a* (L)	0.64
$[NH_4^+]$	–0.31	Assimilation No.	0.40	Cryptophyte Chl *a*	0.63	Cyanobacteria Chl *a* (L)	0.43
K_d (L)	–0.37	Assimilation No. (L)	0.28			Cyanobacteria Chl *a*	0.35
$[NO_x]$	–0.40	pH	0.18			$[NH_4^+]$ (L)	–0.26
$[NO_x]$ (L)	–0.42	Dissolved O_2	–0.71				
K_d	–0.45	Dissolved O_2 (L)	–0.77				

Note: Values in parentheses represent the cumulative contribution of the factors in explaining the total variability associated with the data set. Values (factor loadings) are correlations of the response variable with the common variability represented by the factor. L, "lagged" variables immediately upstream from a sampling site and prior to a sampling date.

Fig. 4. Carbon-specific growth rates (μ_{Chl}) of phytoplankton assemblages as a function of sampling date and location. Values are the mean of three replicates.



($p < 0.01$), with seasonal growth patterns corresponding to spring and fall blooms at all sampling locations (compare Figs. 2 and 4).

The relative importance of 12 physical and chemical variables in explaining the variation in μ_{Chl} was determined (in cumulative order) by a stepwise (forward-selection) multiple regression. Selection of variables for the regression model terminated when the (cumulative) importance of the variables did not further contribute to explaining the variation in μ_{Chl} ($p > 0.05$). The equation:

$$\mu_{Chl}\ (day^{-1}) = 1.7 \times 10^{-3}\ [NH_4^+] + 1.8 \times 10^{-3}\ [PO_4^{3-}] + 8.9 \times 10^{-2}\ (pH) - 5.3 \times 10^{-1}$$

where $[NH_4^+]$, $[PO_4^{3-}]$, and pH are ammonium, dissolved inorganic phosphate ($\mu g \cdot L^{-1}$), and $-\log[H^+]$, respectively, was an adequate descriptor of μ_{Chl} throughout the study period ($p < 0.01$). The three variables used in the model accounted for 41% of the variation in μ_{Chl} ($p < 0.01$). To evaluate the model further, data acquired in the biweekly sampling program for these three variables were used to predict μ_{Chl} for assemblages at each site. Predicted μ_{Chl} corresponded well to measured μ_{Chl} for the four sampling sites ($p < 0.01$; $r^2 = 0.35$ to 0.68). Periods of high growth rates were closely followed (2–4 week lag period) by marked increases in total Chl *a* biomass. Growth rates were positively correlated with phytoplankton assimilation numbers ($r^2 = 0.23$, $p < 0.01$; Fig. 5A), indicating that growth rates were likely associated with photosynthetic efficiency. No significant relationship between μ_{Chl} and productivity ($r^2 = 0.00$, $p > 0.05$; Fig. 5B) was observed. Growth rates were negatively correlated with Chl *a* ($r^2 = 0.15$, $p < 0.01$; Fig. 5C), suggesting that the highest growth rates occurred during periods or locations of low Chl *a* biomass.

Contour plots were used for comparisons of predicted μ_{Chl} with Chl *a* and primary productivity (Fig. 6) over the sampling

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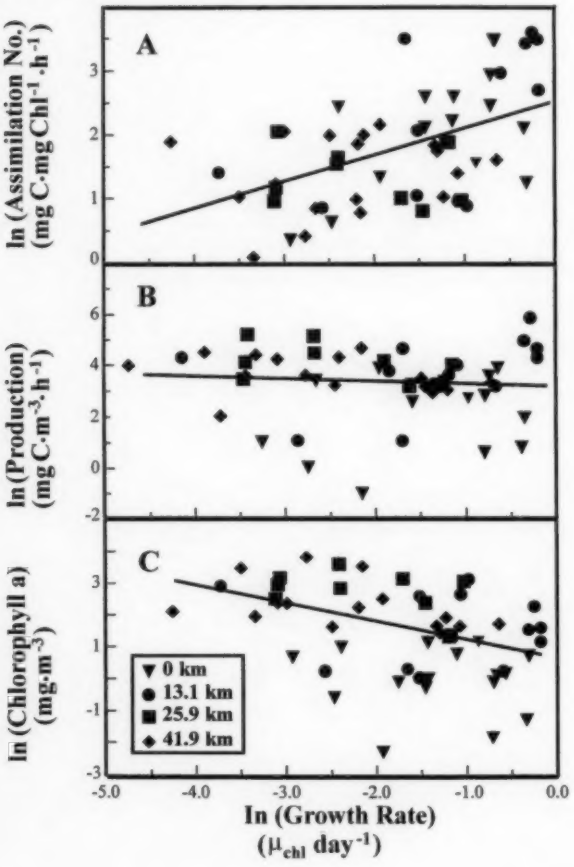
Fig. 5. Assimilation number (biomass-specific production) (A), primary productivity (B), and Chl *a* concentration (C) as a function of carbon-specific growth rate. Values are the mean of three replicates. Lines represent the line of best fit as determined by linear regression analyses ($p \leq 0.05$).



area. Phytoplankton blooms appeared to be initiated upstream and propagated downstream over time. High μ_{Chl} rates generally were restricted to the upper reaches of the estuary. The temporal variability in μ_{Chl} conformed to the spring (April, May, June) and autumn (November, December) bloom periods typically encountered in this estuary (Fig. 4) (Mallin 1994). The high μ_{Chl} observed over the entire length of the estuary during July–September 1994 and August 1995 coincided with periods of high river discharge that resulted in low salinity, water column stratification, and high nutrient loading and ambient concentrations (data not shown). The highest productivity occurred in the middle (10–30 km) reaches of the estuary and biomass, overall, positively correlated with productivity.

Discussion

Although the environmental factors associated with phytoplankton blooms are well documented (Paerl 1988; Glibert et al. 1995), the exact mechanisms and ecophysiological controls on blooms have been difficult to quantify for natural phytoplankton assemblages. Measurements of phytoplankton biomass and production typically are used to characterize the size and duration of a bloom. Such measurements may provide insights into the causes, timing, and (or) sequence of events governing development of phytoplankton blooms. However, the key to understanding bloom phenomena is to examine the spatial and temporal linkages between phytoplankton growth and subsequent increases in biomass and production during bloom proliferation. Consequently, growth rate measurements could prove critical in delineating the stimulatory physical and (or) chemical parameters controlling bloom dynamics.

The PFA identified variables indicative of hydrological forcing (i.e., salinity, conductivity, K_d, NO_x, and NH_4^+ concentrations) to be prominent determinants for phytoplankton accumulation within the Neuse River. In particular, the greater association of variables indicative of riverine discharge were more highly correlated with lagged phytoplankton assemblages than with contemporaneous assemblages (Table 2). Clearly, this association indicates the importance of hydrological forcing in not only the establishment but also the persistence of phytoplankton blooms throughout the estuary. Rudek et al. (1991) and Mallin et al. (1993) have shown that similar factors regulate phytoplankton biomass at the mouth of the Neuse River, near Pamlico Sound.

The highest growth rates and lowest biomass co-occurred within the upper reaches of the estuary. Elevated growth rates, associated with initial stages of blooms, occurred upstream and were manifested as high phytoplankton biomass further downstream. Active bloom development and proliferation occurred in the wider, central portion of the Neuse River. This region is characterized by relatively low turbidity, slow current speeds, and long residence times (>30 days). In the lower reaches of the estuary, blooms achieved the highest biomass. However, during periods of increased flows, elevated rates of phytoplankton growth occurred along the entire length of the estuary. Growth rates were highest during July and August 1994 and August 1995, coinciding with periods of low salinity attributed to high river discharge.

Chlorophyll *a* radiolabeling methods have been used successfully to determine the in situ carbon-specific growth rates of natural phytoplankton assemblages (Goericke and Welschmeyer 1993; Redalje 1993; Hein and Riemann 1995). Growth rates of assemblages in the Neuse River were similar to those reported for other estuarine habitats (Landry and Hassett 1982; Sellner and Kachur 1987; Malone et al. 1988; Cloern 1991; McManus and Ederington-Cantrell 1992). However, phytoplankton growth is influenced by both density-dependent (e.g., self-shading, competition for nutrients, etc.) and density-independent (e.g., salinity, meteorological conditions, nutrient inputs, etc.) factors. Individual taxa exhibit growth rates distinct from the overall assemblage growth rate (Fahnenstiel et al. 1995). Consequently, Chl *a* specific growth rates represent integrated values for a variety of taxa constituting the assemblage.

Growth rates were negatively correlated with Chl *a* concentrations and positively correlated with phytoplankton assimilation numbers. Stormwater inflows provide contemporaneous nutrient loading and dilution of biomass within estuarine systems draining agricultural lands (Klarer and Millie 1989). Such nutrient enrichment would allow for an increased physiological capacity for phytoplankton growth within the Neuse River

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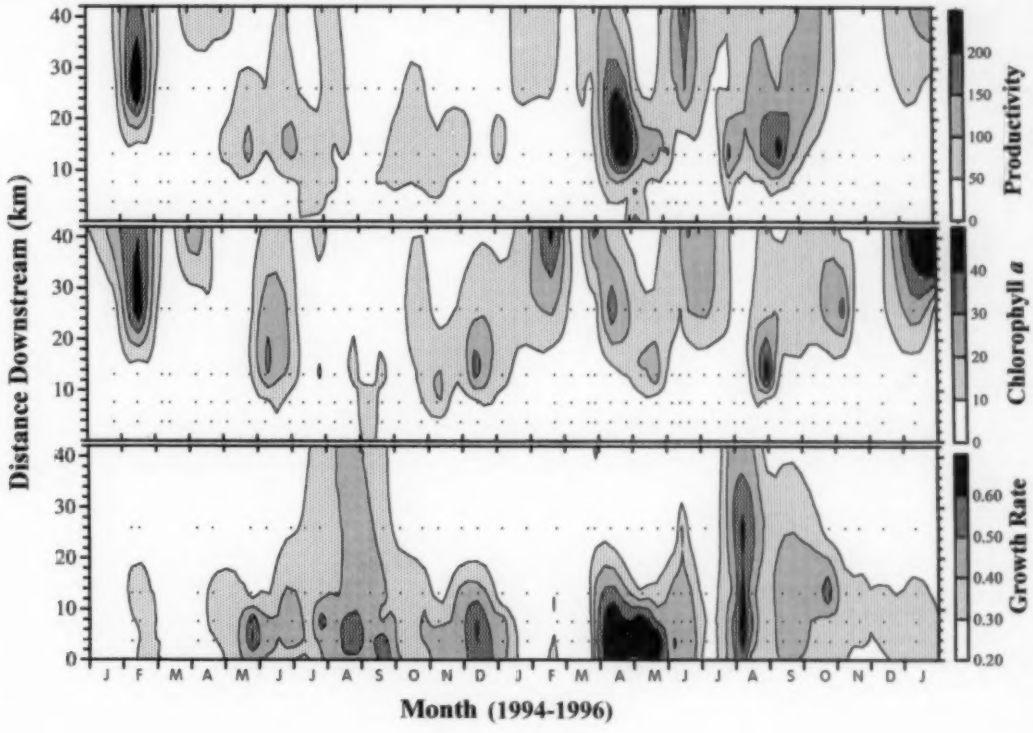
Fig. 6. Spatiotemporal mosaics of predicted phytoplankton community carbon-specific growth rates (calculated from regression model) (day^{-1}), Chl *a* concentrations ($mg \cdot m^{-3}$), and phytoplankton productivity ($mg\ C \cdot m^{-3} \cdot h^{-1}$).



Estuary (Klarer and Millie 1994). During periods of low phytoplankton biomass, the effects of density-dependent factors upon phytoplankton growth would be reduced, and in the presence of sufficient nutrients and light, phytoplankton would exhibit elevated growth rates and photosynthetic efficiencies. This growth would be expected to continue as assemblages are transported downstream and into the less turbid and slower flowing lower reaches of the estuary.

The combined results of the PFA and growth rate regression suggest a dynamic interplay of factors control bloom dynamics in the Neuse River. Phytoplankton biomass (Chl *a*) is clearly associated with hydrological factors (salinity, light, N loading), while growth rate is more closely correlated with ammonium, pH, and phosphate levels. However, a straightforward cause and effect relationship between these variables and phytoplankton biomass and (or) growth rate cannot be fully developed here. Rather, these variables may be surrogates for other (unmeasured) processes that regulate phytoplankton growth within the Neuse River. For example, NO_x and NH_4^+ loadings are indicative of surface runoff and subsurface interflow, respectively, following storm events (Klarer and Millie 1989). Additionally, elevated pH and NH_4^+ and PO_4^{3-} concentrations are associated with nutrient regeneration processes and stratification (Christian et al. 1991; Rizzo et al. 1992; Paerl et al. 1995). Under such conditions, residence times are longer and vertical mixing is reduced, further supporting bloom events. In the lower reaches of the estuary, high zooplankton grazing rates (up to 40% of phytoplankton production) have a dual effect of reducing phytoplankton biomass while enhancing nutrient (PO_4^{3-} and NH_4^+) concentrations, regeneration, and cycling in the water column (Mallin and Paerl 1994).

The contour plots provide temporal mosaics of phytoplankton growth rates, Chl *a* biomass, and primary productivity and illustrate the spatial relationships of the assemblages. The mosaics of phytoplankton biomass (Chl *a*) and productivity are similar to those previously reported for the Neuse River Estuary during 1983–1987 (Boyer et al. 1993). Productivity generally coincided with phytoplankton biomass; however, phytoplankton assimilation numbers were lowest in the lower portion of the estuary, indicating a decrease in photosynthetic efficiency. This suggests phytoplankton were growing at suboptimal rates, most likely the result of environmental stress and (or) some form of physical or chemical limitation.

The phytoplankton community in the Neuse was relatively diverse, with periodic blooms of cyanophytes, chlorophytes, cryptophytes, diatoms, and dinoflagellates (Fig. 2). Blooms in the upper reaches of the study area (0–3.7 km) are composed primarily of cyanophytes, chlorophytes, and cryptomonads while the lower reaches (7.6–41.9 km) experience dinoflagellate, cryptomonad, and occasional chlorophyte blooms. Phytoplankton assemblages in the Neuse River system are considered to be nitrogen limited (Paerl 1987; Rudek et al. 1991; Boyer et al. 1994); nitrogen uptake rates are variable, but NH_4^+ is the preferred nitrogen source, even in the presence of high NO_3^- concentrations (>30 μM; Boyer et al. 1994). Light may be a secondary, but significant, determinant

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regulating overall phytoplankton biomass. The distinct Chl *a* : accessory pigment ratios exhibited by dinoflagellates and cryptophytes during bloom events (see Figs. 3C and 3D) suggest that these species may alter their pigment composition (photoacclimate) to maximize light absorption (McManus and Ederington-Cantrell 1992; Millie et al. 1993).

The low growth rates and assimilation numbers in the lower reaches of the estuary may reflect physiological stress and (or) mortality of oligohaline phytoplankton species encountering mesohaline conditions (Christian et al. 1984). However, phytoplankton species in this estuary typically have a wide salinity tolerance (Christian et al. 1984; Mallin 1994). The dynamic salinity gradient (0–20‰) in the lower estuary may select for specific bloom-forming taxa. Nevertheless, the "players" within assemblages may change, but the underlying ecophysiological processes promoting and sustaining blooms appear consistent.

If residence times are long enough, phytoplankton export from the Neuse River Estuary into Pamlico Sound is minimized. During bloom senescence, algal cells sink into bottom waters and are delivered to the sediment surface. This excessive carbon loading and deposition, coupled with elevated rates of microbial degradation, benthic or planktonic respiration, and chemical oxygen demand, produce zones of hypoxia or anoxia within bottom waters. Anoxic conditions enhance benthic processes that deliver nutrients to the water column. This scenario often follows extensive phytoplankton blooms in the Neuse River. For example, during July and August of 1995, phytoplankton growth rates were higher than during the same period in 1994. The resulting increase in phytoplankton biomass coincided with large-scale anoxia or hypoxia along the length of the estuary (August–September 1995) (Paerl et al. 1995).

Despite the increasing areal coverage, duration, and frequency of harmful algal blooms, little is actually known about the mechanisms and processes forcing bloom initiation. This informational void frustrates water-quality managers in search of effective strategies for predicting and potentially arresting blooms in waters undergoing dynamic (mostly increasing) alterations in nutrient loading (Paerl et al. 1995). This study suggests that nutrient loading and low phytoplankton biomass promote elevated growth rates in the upper reaches of the Neuse River Estuary; growth that continues as phytoplankton are transported into the lower reaches where low mixing rates, low turbidity, and high residence times lead to biomass accumulation. Reductions in nutrient loading would therefore appear to be the only feasible constraint strategy for reducing the frequency and magnitude of blooms. A 30% reduction in overall riverine nitrogen has been targeted for the Neuse Estuary and the results of this research indicate that this is a prudent and potentially beneficial strategy. Management strategies must focus additionally on phytoplankton growth and associated bloom potentials in the upper, riverine reaches of the estuary.

Acknowledgments

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Effect of prey size on the estimation of behavioral visual resolution of bluegill (*Lepomis macrochirus*)

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Abstract: Unlike larger conspecifics (>35 mm SL), behavioral visual resolution of young-of-the-year bluegill (*Lepomis macrochirus*) (11–32 mm SL) measured as visual angle is not independent of prey size. Visual angles based on the maximum location distances (MLDs) for bluegill ≤32 mm SL increased directly with prey size and differed significantly among three prey size categories (*Daphnia pulicaria*: carapace length 2.3, 1.1, and 0.8 mm). Visual angles of small bluegill viewing their preferred prey were approximately half those for fish viewing the largest prey and indicate that the behavioral visual resolution of small bluegill was previously underestimated. Errors in prey size measurements have a much larger effect on visual angle than do errors in reaction distance. In most instances, MLDs of small bluegill to large prey were significantly longer than were MLDs to small- and medium-sized prey. MLDs for the smallest prey were about 70% those for the largest prey; the difference between the MLDs within a fish size category is much less than predicted if visual angle is independent of prey size. Poor visual and locomotory capabilities of small bluegill probably contribute to the comparatively small differences in reaction distances to small versus large prey.

Résumé : À la différence de ce qu'on observe chez les conspécifiques de plus grande taille (>35 mm LS), la résolution visuelle comportementale des jeunes de l'année de crapet arlequin (*Lepomis macrochirus*) (11–32 mm LS) mesurée en tant qu'angle visuel, n'est pas indépendante de la taille des proies. Les angles visuels calculés à partir des distances maximales de localisation (MLD) pour les crapets ≤32 mm LS augmentent directement en fonction de la taille des proies et diffèrent de façon significative entre trois catégories de taille des proies (*Daphnia pulicaria* : longueur de carapace de 2,3, 1,1 et 0,8 mm). Les angles visuels des petits crapets observant leur proie favorite étaient inférieurs de moitié environ à ceux des poissons observant les proies les plus grosses, ce qui indique qu'on a jusqu'à maintenant sous-estimé la résolution visuelle comportementale des petits crapets. Les erreurs dans les mesures de la taille des proies ont un effet beaucoup plus grand sur l'angle visuel que les erreurs dans la distance de réaction. Dans la plupart des cas, les MLD des petits crapets par rapport aux proies de grande taille étaient significativement plus longues que les MLD par rapport aux proies de taille petite et moyenne. Les MLD par rapport aux proies les plus petites correspondaient à environ 70 % de celles observées avec les proies les plus grosses; la différence entre les MLD au sein d'une catégorie de taille de poissons est nettement inférieure à celle qui serait prévue si l'angle visuel était indépendant de la taille des proies. Les faibles capacités visuelles et locomotrices des petits crapets ont probablement un effet sur les différences comparativement faibles dans les distances de réaction à l'égard des proies de petite taille et de grande taille.
[Traduit par la Rédaction]

Introduction

Understanding the role of visual resolution in predator–prey interactions has important implications for testing foraging and community-level models for freshwater and marine systems. The retina of a teleost fish grows throughout the animal's lifetime (Müller 1952; Johns and Easter 1977; Hairston et al. 1982; Easter 1992) and the changing visual capabilities of fish are central to the estimation of encounter probabilities required for most diet choice and habitat switching models where the effective availability of prey in the environment is a function of visibility (Werner and Hall 1974, 1988; O'Brien, 1979; O'Brien et al. 1976; Mittelbach 1981, 1984; Eggers 1982; Aksnes and Giske 1990, 1993). Young-of-the-year are typically the most abundant age-class of fish populations (Beard 1982) and can potentially play an important role in structuring zooplankton communities through size-selective predation. Ontogenetic changes in the visual system will greatly influence prey detection ability (Baerends et al. 1960; Blaxter 1975; Otten 1981; Hairston et al. 1982; Breck and Gitter 1983; van der Meer and Anker 1984; Li et al. 1985; Fernald 1988; Sivak 1990; Walton et al. 1994) and it is clear that while retinal growth is central, another unidentified factor plays a critical role when fish are small.

Behavioral and anatomical measures of visual resolution that are highly correlated in bluegill (*Lepomis macrochirus*) >30 mm standard length (SL) (Hairston et al. 1982; Breck and Gitter 1983; Li et al. 1985) are less strongly correlated in small sunfish (<20 mm SL; Walton et al. 1994). The lower extent of association between behavioral and anatomical measures of

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visual resolution indicates that, in addition to ontogenetic changes of cone cell spacing and lens focal length, other factors influence the prey detection abilities of small bluegill. Prey detection by small bluegill foraging on 2-mm *Daphnia* requires images that are greater than two times the average intercone spacing on the retina, and the proportion of prey detections with an image size greater than twice the intercone interval declines with increasing fish size (Walton et al. 1994). Such behavioral observations suggest that the image of the prey on the retina might be blurred in very small sunfish; comparatively poor lens quality, inadequate accommodative mechanisms that move the lens and focus the image on the retina, or lower neuronal connectivity (Blaxter 1975; Wagner 1978; Otten 1981; Fernald 1988; Sivak 1990) might cause the blurred images. Cognitive factors, such as learning (Meyer 1986), prey recognition (Hughes 1979), and prey size selectivity, might also affect the size-related changes of behavioral visual resolution. Our study examines the effect of prey size on estimates of behavioral visual resolution for bluegill foraging on crustacean prey (*Daphnia pulicaria*).

Materials and methods

Bluegill larvae were collected off a nest in Rainbow Springs Lake, Waukesha County, Wis., U.S.A., and reared in the laboratory on daily additions of a mixed assemblage (i.e., *Synchaeta* spp., *Polyarthra* sp., *Asplanchna* sp., *Bosmina longirostris*, *Ceriodaphnia* sp., *Leptodiaptomus ashlandi*, and *Diacyclops thomasi*) of nearshore Lake Michigan zooplankton. Juvenile bluegill (>25 mm SL) were caught with a seine in Lulu Lake (Walworth County, Wis.) and maintained in the laboratory on a daily ration of Lake Michigan zooplankton and freeze-dried *Tubifex* worms.

Experiments were carried out in an observation tank (bottom dimensions 21 × 14 cm; water depth 2 cm) with a grid of 1-cm squares that was affixed to a sheet of white Plexiglas on the bottom of the aquarium. To determine the visual angles for bluegill >18 mm SL where reaction distances might approximate, or exceed, the dimensions of the small aquarium, the reaction distances were measured in a larger aquarium (bottom dimensions 60 × 30 cm) at a water depth of 4 cm. The aquaria were wrapped with white paper to minimize distractions and provide the fish a uniform visual background. Illumination was provided by three 40-W warm-white fluorescent lamps (λ_{max} = 550 nm) giving ambient light levels at the water's surface of 4.32 μeinsteins·s^{-1}·m^{-2}.

Prey detection by bluegill is indicated by a reorientation of the long axis of the body towards the prey (Vinyard and O'Brien 1976). Reorientation is typically followed by rapid swimming to the location of the prey and capture. Reaction distance is defined as the distance between predator and prey at which the predator exhibits this characteristic orientation behavior. Visual angle is defined as the angle at the fish's eye subtended by the prey at detection and is a function of prey size and the reaction distance.

The reaction distances of bluegill were measured from videotapes of fish foraging. The video camera was positioned 88 cm above the water's surface. Three fish of similar size were placed into the observation tank because individual fish were skittish and did not readily feed. The observer watched the fishes' behavior on a television monitor that was positioned outside the visual field of the fish. Laboratory-reared *Daphnia* were sieved and then sorted by hand into three size categories (mean lengths ± SE: 0.76 ± 0.03, 1.1 ± 0.1, and 2.3 ± 0.1 mm). For each observation period, the body size either of a representative sample (N = 25–30 individuals) for each prey size-class or of individuals was measured, separated by body size into tissue culture wells, and then used in the experiments. Individual prey were placed into the aquarium by a pipet at a position in the tank opposite that occupied by the fish. Sham introductions were routinely done to avoid the fish associating the pipet with food. Reaction distance (d) was measured by calculating the distance between the x,y-coordinates of the fish's head at orientation to the prey and at the attack on the prey. The visual angle (θ) was calculated as

$$(1) \qquad \theta = 2\tan^{-1}(h/2d)$$

where h is prey size. Hairston et al. (1982) assumed that prey size was equivalent to the average diameter of a sphere having the same volume as an oblate spheroid with its major axis being *Daphnia* length and width and its minor axis equal to *Daphnia* thickness; prey size is equal to the carapace length multiplied by 0.64. This proportion also approximates the fraction of the *Daphnia* carapace length (excluding the tail spine) that is occupied by the pigmented digestive tract.

The maximum location distance (MLD) is a better behavioral measure of visual capabilities than is the mean reaction distance for fish that use a saltatory search strategy (O'Brien et al. 1990). The MLD is the outer boundary of the distribution of pursuit length within which 90% of the pursuits fall (O'Brien and Evans 1991). The MLD was calculated using the longest 10% of the reaction distances for each prey size – fish length category.

The angle subtended by the prey at the fish's eye at the moment of detection was compared among the three *Daphnia* size categories across the range of fish size-classes by ANCOVA using ln-transformed SL as a covariate. To satisfy the assumptions for a straight-line ANCOVA model, visual angles were also ln-transformed prior to analysis. For each prey size category, visual angles for the entire range of bluegill were then examined quantitatively by transforming SL to natural logarithms and fitting ln-transformed visual angle to this variable using least squares linear regression. Only visual angles calculated from the longest 10% of the reaction distance distributions for each of the prey size – fish length categories were used in these analyses.

To examine how prey selectivity of bluegill changes as fish grow, we compared prey selection with that expected from random encounters of fish with zooplankton prey. Preference is any deviation from random sampling of the prey (Chesson 1983). The preference measure, α_i (Chesson 1983), was calculated as

$$(2) \qquad \alpha_i = \frac{r_i/n_i}{\sum_{j=1}^{m} r_j/n_j}, \quad i = 1, \ldots, m$$

where n_i is the expected number of prey type i present in the diet assuming random encounters with the prey and r_i is the number of items of food type i in the diet. In contrast with the reaction distance experiments where individual prey were used, prey densities in the prey selection experiments ranged from 9 to 20·L^{-1}. The expected diets for each fish size category (11, 14, 16, 29, 38, 60, and 76 mm SL) studied by Li et al. (1985) and Walton et al. (1992) were computed using Wetterer and Bishop's (1985) model for prey selection based on apparent size. The expected distributions of attacks were corrected for truncation of the visual field by shallow water depth using $2\pi a(d^2 - a^2/3)$ (Werner and Hall 1974) where d is reaction distance and a is equal to one half the depth of the experimental environment.

Results

The visual angles of bluegill for all prey size categories decreased nonlinearly with increasing fish size (Fig. 1) and were directly related to prey size (Table 1). Visual angles for bluegill viewing different-sized prey differed significantly (ANCOVA of prey size on ln-transformed θ using ln-transformed SL as the covariate; prey size: $F_{2,97}$ = 142.4, P < 0.0001; homogeneity of slopes: $F_{2,95}$ = 0.363, P > 0.697). Behaviorally determined visual angles for bluegill between 11 and

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32 mm SL decreased by 52 and 103 minutes of arc for the smallest and largest prey size categories, respectively, used in our experiments.

Visual angles based on the MLD differed significantly among the prey size categories, with greatest detection capability for the smallest prey (Table 1). The difference in visual angle between the small and large prey size categories is approximately 50% (mean ± SE: 0.50 ± 0.11). Visual angles for 25-mm fish differ from this trend; however, the calculations are based on only two observations per prey size category.

For each bluegill size category, MLD increased directly as a function of prey size (Table 1). MLDs within a particular bluegill size category to the smallest prey were on average 71% of those for the largest prey ($MLD_{small\ prey}/MLD_{large\ prey}$, mean ± SE: 0.71 ± 0.13). Reaction distances of bluegill to the largest prey were significantly longer than to small- and medium-sized prey (Tukey's HSD; $P > 0.05$). MLD increased by approximately 12-fold as bluegill grew from 11 to 32 mm SL.

Even though the ratio of the mean reaction distance to small versus large prey ($RD_{small\ prey}/RD_{large\ prey}$, mean ± SE: 0.70 ± 0.08) was similar to that for the MLD, mean reaction distances of bluegill >25 mm SL differed significantly among prey size categories (Tukey's HSD; $P < 0.05$) (Table 1). For bluegill ≤25 mm SL, mean reaction distance did not differ significantly among prey size categories (Table 1). In contrast with the mean reaction distance, the MLD for the largest and smallest prey differed significantly for 75% of the fish size-classes examined.

The mean reaction distance, the minimum reaction distance, and the variance around the mean reaction distance increased directly with fish size (Fig. 2). The reaction distances of 11-mm bluegill ranged narrowly from 1 to 5 cm whereas the reaction distances of 30- to 32-mm bluegill ranged broadly from 11 to 46 cm.

Prey selection by the three smallest and two largest bluegill size-classes differs significantly from that predicted from random encounters with prey (χ^2 tests; $P < 0.05$, df = 2). Bluegill ≤16 mm SL select significantly more small prey, and fewer intermediate and large prey, than expected from prey visibility (Fig. 3). Preference values of bluegill <16 mm SL were high ($\alpha_i > 0.7$) for small prey and low ($\alpha_i < 0.2$) for intermediate and large prey. Conversely, bluegill ≥60 mm SL preferentially chose the largest *Daphnia* and consumed fewer small prey than is expected from random encounters. *Daphnia* of intermediate size were attacked as encountered (α_i between 0.3 and 0.35). Prey selection by bluegill of intermediate size (29.3 and 38 mm SL) did not differ significantly from that expected on the basis of random encounters with prey (χ^2; $P > 0.05$, df = 2); preference values of intermediate-sized bluegill for the three prey size categories were comparatively similar to those of smaller and larger conspecifics (Fig. 3).

Discussion

The independence of visual angle from prey size is essential when making intraspecific (i.e., among fish size-classes) or interspecific comparisons of the behavioral visual resolution of fish where the prey size used in experiments differs (Wanzenböck and Schiemer 1989; Browman et al. 1990; Wanzenböck 1992; Wahl et al. 1993; Walton et al. 1994). Behaviorally determined visual angles are of similar magnitude for a particular fish size-class reacting to prey of the same apparent size, provided that prey do not exhibit size-related differences in coloration, movement patterns, and morphology. The visual angle subtended by the prey at detection is independent of prey size in bluegill >30 mm SL (Hairston et al. 1982; Breck and Gitter 1983; Li et al. 1985). Visual angles were calculated using the mean or median reaction distance in the aforementioned studies on bluegill (Hairston et al. 1982; Breck and Gitter 1983; Li et al. 1985); visual angles based on the MLD for bluegill >45 mm SL do not differ appreciably among prey size categories (see Hairston et al. 1982). For bluegill ≤45 mm SL (Hairston et al. 1982), visual angles based on the MLD differ among prey size categories; although visual angles for fish viewing small prey tend to be smaller than for the largest prey, the differences are not strongly related to the *Daphnia* size categories. The data presented here indicate that behaviorally determined visual angles of small bluegill (≤32 mm SL) are not independent of prey size.

Because visual angle increases directly as a function of prey size, visual resolution of small fish will be underestimated when large prey are used in behavioral experiments. Visual resolution is inversely related to visual angle. To discern individual *Daphnia* and reduce errors caused by parallax in the estimation of reaction distance during the analysis of videotapes (i.e., use of a long working distance between the video

Fig. 1. Behaviorally based estimates of visual resolution of juvenile bluegill as a function of fish SL. The visual angles were calculated from reaction distances of fish ($N = 36$) to three sizes of *Daphnia*: small (~0.8 mm, diamonds), medium (~1.1 mm, triangles), and large (~2.3 mm, plus signs). The data for each fish size are spread out horizontally to facilitate illustration. Least squares regressions of visual angle, θ, in minutes of arc for the longest 10% of the reaction distances (MLDs) for a particular prey size – fish size-class category on fish length (SL) in millimetres for each prey size category are shown: small prey (dotted curve), $\ln \theta = 8.33 - 1.90 \ln SL$ (SE of slope = 0.12, $N = 33$, $F = 248.81$, $P < 0.0001$); medium prey (dashed–dotted curve), $\ln \theta = 8.99 - 2.02 \ln SL$ (SE of slope = 0.11, $N = 34$, $F = 318.58$, $P < 0.0001$); large prey (solid curve), $\ln \theta = 9.17 - 1.93 \ln SL$ (SE of slope = 0.06, $N = 34$, $F = 895.46$, $P < 0.0001$).

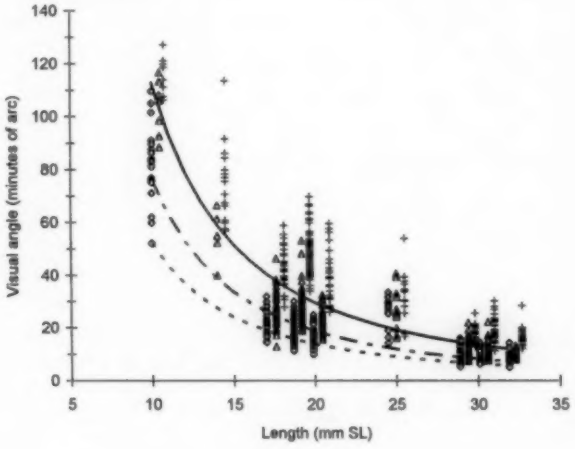

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Table 1. Ontogenetic change in the visual angles based on the MLDs and mean reaction distances of bluegill (SD in parentheses).

SL	*N*	Visual angle (θ, minutes of arc)			MLD (cm)			Mean reaction distance (cm)		
(mm)	(*S*, *M*, *L*)	Small	Medium	Large	Small	Medium	Large	Small	Medium	Large
11	3, 2, 2	58.0 (5.2) *a*	90.6 (3.0) *b*	116.9 (1.0) *c*	2.2 (0.4) *a*	2.2 (0.1) *a*	3.5 (0.1) *b*	1.8 (0.5) *a*	1.8 (0.3) *a*	3.0 (1.4) *a*
14	—, 1, 3		39.9	58.0 (1.6)		6.2	7.4 (0.2)		7.0 (1.5) *a*	9.7 (0.8) *a*
18	4, 5, 4	15.9 (1.2) *a*	20.2 (1.3) *b*	30.7 (1.9) *c*	10.8 (0.8) *a*	14.0 (2.9) *ab*	15.2 (1.0) *b*	7.9 (1.6) *a*	9.7 (2.4) *a*	11.0 (2.2) *a*
19	10, 7, 7	13.2 (1.2) *a*	18.0 (3.2) *b*	37.1 (2.1) *c*	13.1 (1.2) *a*	13.4 (0.8) *a*	13.7 (0.8) *a*	9.2 (2.0) *a*	9.4 (1.8) *a*	10.5 (2.0) *a*
20	4, 4, 4	11.5 (1.1) *a*	16.7 (1.0) *b*	27.5 (1.0) *c*	14.9 (1.5) *a*	15.3 (1.0) *a*	18.6 (0.7) *b*	10.6 (2.5) *a*	11.2 (2.1) *a*	13.2 (3.3) *a*
25	2, 2, 2	13.4 (1.5) *a*	16.6 (0.7) *a*	17.4 (0.8) *a*	8.7 (0.3) *a*	13.2 (0.6) *b*	16.9 (1.2) *c*	7.8 (1.0) *a*	8.6 (2.7) *a*	11.5 (4.1) *a*
30	4, 4, 4	6.0 (0.4) *a*	8.1 (0.7) *b*	13.3 (0.6) *c*	29.9 (1.9) *a*	31.5 (2.5) *a*	40.0 (1.6) *b*	19.0 (5.7) *a*	22.2 (5.6) *a*	32.3 (5.4) *b*
31	4, 4, 4	6.6 (0.3) *a*	8.3 (0.6) *b*	12.7 (1.1) *c*	25.9 (1.0) *a*	30.4 (2.3) *a*	41.6 (3.7) *b*	18.4 (4.4) *a*	22.9 (5.1) *b*	28.4 (7.2) *c*
32	5, 4, 4	6.4 (0.9) *a*	8.2 (0.3) *b*	13.5 (1.1) *c*	29.1 (4.0) *a*	30.0 (1.0) *a*	39.4 (3.2) *b*	20.5 (4.0) *a*	24.6 (3.2) *b*	31.9 (4.7) *c*

Note: *N* is the sample size for calculations of visual angle and the MLD where *S*, *M*, and *L* are the number of observations for small, medium, and large prey size categories, respectively. Visual angles, MLDs, or mean reaction distances for the three size-classes of *Daphnia* followed by the same letter do not differ significantly (Tukey's HSD; $P > 0.05$) within a particular fish size category.

Fig. 2. Distribution of reaction distances among bluegill size categories. The distributions are moving averages of three (bluegill ≤20 mm SL) or 10 points (bluegill >20 mm SL) for equivalent numbers of attacks on the three prey size categories. The reaction distances for 18- to 20-mm bluegill are summed and plotted as 19 mm. The reaction distances for 30- to 32-mm bluegill are summed and plotted as 31 mm.

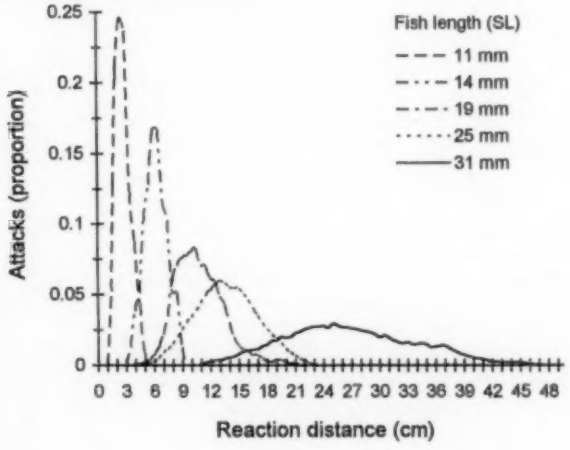
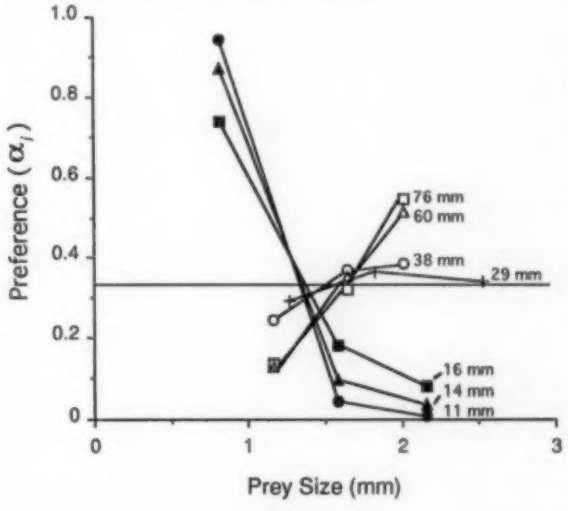


Fig. 3. Change in prey preference (α_i) of bluegill during growth. The preferences of fish in six size-classes offered three sizes, *i*, of *Daphnia* are illustrated. Preference values were calculated from data reported in Li et al. (1983) and Walton et al. (1992). $\alpha_i = 0.33$ is the value for no selectivity; above this value, prey are attacked in greater proportion than encountered and below this value are attacked in lesser proportion than encountered.



camera and the fish), large prey (2-mm *Daphnia*) were used in reaction distance trials by Walton et al. (1994). The visual angles for all bluegill to the largest *Daphnia* used here are of comparable magnitude with those reported by Walton et al. (1994); however, the results presented here indicate that the behavioral visual resolution of small bluegill was previously underestimated by approximately twofold (Fig. 1).

The difference in visual angles across the fish size-classes used here for the smallest and largest prey size categories is approximately 50%. It is unlikely that measurement errors caused a nearly 50% difference in visual angles for fish within a particular size-class viewing the smallest versus the largest prey. The magnitude of errors in visual angle is inversely related to prey size and reaction distance. Errors in prey size measurements have a much greater effect on visual angle estimates than do errors in reaction distance. If prey size is under- or overestimated by 0.1 mm, then the error in visual angle using the smallest prey size category is approximately three times larger than when using the largest prey. When 0.79-mm prey are used in experiments, visual angle changes by approximately 13%·0.1 mm^{-1}. If 2.34-mm prey are used, visual angle changes by only 4%·0.1 mm^{-1}. At visual angles of 58′, a 0.1-mm error in the size of small prey changes visual angle by 7.3′ whereas a similar error in the size of large prey changes visual angle by 2.5′. Errors in reaction distance of 0.1 cm change visual angle by a comparatively small and decreasing amount as visual angle declines. This difference is only about 3 and 1% for small and large prey, respectively, when visual angle is 58′ and <1% for both prey categories when visual angle is <13′.

Several aspects of our experimental conditions may not duplicate natural conditions, but as long as the effects are equivalent

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across prey size categories, our conclusions regarding the effects of prey size on estimates of behavioral visual resolution should remain unchanged. The cues commonly used by visual predators for prey recognition include size, movement, and shape (McFarland 1985). The probability that a prey will be detected by a predator is influenced by its angular size and velocity, the degree of contrast with the background, and the general level of illumination (McFarland 1985). Fluorescent lights do not duplicate the distribution and range of electromagnetic spectral components found in nature or provide all wavelengths (e.g., near ultraviolet) possibly used by fishes to detect prey (Cameron and Pugh 1991; Guthrie and Muntz 1993). The white background used in our experiments is likely to enhance prey visibility relative to that under natural conditions where the contrast between the background and the prey is less. Visual angles based on the MLD (Table 1) are by definition smaller than those reported previously based on the mean or median reaction distance to prey of equivalent size (Hairston et al. 1982; Breck and Gitter 1983; Li et al. 1985). Yet, visual angles calculated using the mean reaction distance for 30- to 32-mm SL fish(~18′, Table 1) are still smaller than those of 37-mm SL bluegill (27′) used by Hairston et al. (1982). Visual angles (calculated using the MLDs) of 30- to 32-mm SL bluegill viewing a 0.8-mm *Daphnia* were nearly 6′ (Table 1) and approach the minimum visual angle found among aquatic predators. Guthrie and Muntz (1993) found that aquatic predators (i.e., fish, marine mammals, and cephalopods) do not have visual acuities better than 4′ and suggested that visual acuity better than 4–5′ serves no useful purpose in aquatic environments where high spatial frequencies (i.e., the fine detail of objects being viewed) are heavily attenuated.

To date, planktivorous fish have not been shown to estimate absolute prey size; yet, prey selection often differs from that predicted by apparent size alone (Fig. 2). Wetterer (1989) suggested that sunfish use apparent size to direct their attention to a prey item when prey encounters are simultaneous and then evaluate actual prey size. Ewert and Burghagen (1979) found that at about 2 weeks after metamorphosis, midwife toads (*Alytes obstetricans*) use apparent size to judge prey size and that 6 months later, toads make prey size judgments independently of the visual angle. Object size constancy has also been observed other anurans (*Rana pipiens* (Ingle 1968) and *Bufo bufo* (Ewert and Gebauer 1973). To estimate absolute prey size, fish must use retinal size and an estimate of distance. Several mechanisms for how fish perceive distance have been suggested: parallax while moving through the water (assumes sunfish search during repositioning movements; Li et al. 1985), binocular disparity (Wetterer 1989), and a combination of stimulus recognition (e.g., learned allometric relationships for morphology) and visual angle (Tegeder and Krause 1995). The ability of fish to estimate distance as travel time, or the inverse of encounter rate, is important in optimal foraging models where prey encounters are simultaneous (Stephens and Krebs 1986).

In situations where more than one prey is visible at a particular time, a progressive shift in prey preference to larger prey occurs as planktivorous bluegill grow (Fig. 3). Small bluegill (<17 mm SL) preferentially attack *Daphnia* similar in size to the smallest size-class used here (Fig. 3), but small bluegill will also attack *Daphnia* in the largest size-class when they are the only prey available (Walton et al. 1992). Larger prey are incorporated into the diet as the fish grow and capture efficiency increases. Prey size preference shifts markedly for bluegill between 10 and 29 mm SL (Fig. 3). At 29 and 38 mm SL, bluegill can effectively capture prey in each size-class and attack *Daphnia* as encountered when prey density is between 9 and 20 individuals·L^{-1}. Larger bluegill (i.e., >38 mm SL) exhibit preferences for the largest *Daphnia* size-class.

Our visual angle results suggest that small bluegill are significantly better at detecting their preferred small prey than they are at perceiving large prey; however, the difference between the MLDs to the smallest and largest prey is smaller than expected under the assumption of independence of visual angle on prey size. If visual angle is independent of prey size, then the MLD to the smallest prey should be approximately 34% of the MLD to the largest prey. On average, MLDs to the smallest prey were only about 70% of the MLDs to the largest prey. Reaction distances of small bluegill to prey of different sizes do not differ as much as do those of larger conspecifics for which visual angle is independent of prey size.

Poor visual and locomotory capabilities of small bluegill probably contribute to the comparatively small differences in reaction distances to small versus large prey. Bluegill exhibit saltatory search behavior where repositioning movements occur between locomotive pauses (Janssen 1982; Ehlinger and Wilson 1988; O'Brien et al. 1989). Prey selection occurs primarily during the pauses. The geometry of the search space and the movement patterns of fish utilizing a saltatory search strategy change with environmental conditions, prey type (O'Brien et al. 1989), and ontogeny (Browman and O'Brien 1992*a*, 1992*b*). Although visual resolution is initially poor, vision improves rapidly in young-of-the-year bluegill (Walton et al. 1994). The reaction distance and the distance over which attacks are carried out increase directly with fish size. Browman and O'Brien (1992*a*) found, however, that white crappie (*Pomoxis annularis*) larvae did not efficiently moderate the distance between locomotive pauses; the distance between pauses was longer than the location distance. Search behavior became more energetically efficient as fish grew. The swimming movements of large juveniles (>20 mm SL) are about 40% of the MLD and are directly related to prey size (O'Brien et al. 1989).

Several factors might lessen the distance over which a small bluegill will react and initiate an attack on prey. Small bluegill search comparatively small visual volumes, may require a minimum distance before initiating an attack because of poor attack ability, and may be learning to recognize prey. Further, attacks by small fish are more deliberate than those of larger juveniles, and the attack posture (i.e., sinuous posture) of larvae requires that the fish be located close to prey before initiating an attack (Browman and O'Brien 1992*a*). The variance around the mean reaction distance and the minimum reaction distance increased directly with bluegill size (Fig. 2).

Larval fish also often track the movement of individual prey (Browman and O'Brien 1992*a*), and while this may give the fish an indication of distance and (or) prey size, a small bluegill may not immediately exhibit the characteristic reaction behavior when it sees a prey. The fact that the minimum reaction distance increases directly as a function of fish size suggests that bluegill might be assessing prey location prior to the terminal pause before initiating an attack, perhaps at the

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penultimate pause or during transits. That is, if a 31-mm bluegill were searching for prey only during pauses, then by chance alone one might expect the fish to pause within 11 cm of a prey. Yet, reaction distances <11 cm were not observed for 31-mm bluegill.

Are our findings biologically significant or merely a statistical error (i.e., a Type II error)? Use of the MLD reduces sample size and the power of the statistical comparisons. The power of the comparisons of MLDs to the largest prey versus the two other prey categories for bluegill >20 mm SL is >0.75. The power of similar comparisons for the mean reaction distance was >0.8 for all bluegill size catgeories. Comparisons of MLDs to small- versus medium-sized prey are generally less powerful (power <0.5) as are comparisons for the mean reaction distance (power ranged from 0.1 to 0.99). The statistical significance of the comparisons is opposite that expected from a reduction in sample size: the mean reaction distance to different-sized prey did not differ significantly within fish size categories for bluegill <25 mm SL whereas the MLDs to the largest versus smallest prey differed significantly. Even though the ratio of the mean reaction distance to the smallest versus largest prey is approximately 0.7 across the fish size-classes, the difference between reaction distances to each of the three prey size categories increases directly as fish grow (Table 1). Increasing sample size would aid in distinguishing among the mean reaction distances of bluegill <25 mm SL to the three prey size categories; however, the power of overall statistical comparison of visual angles (based on the MLD) among prey size categories is high, about 0.89.

We also assumed that each reaction distance measurement represents an independent observation. Pseudoreplication in behavior studies can underestimate *P*-values (Lombardi and Hurlbert 1996). However, if we assume a worst-case scenario where MLD measurements are based on only one fish per prey size category at each fish size-class ($N = 26$), then visual angles are still significantly different among prey size-classes (ANCOVA of prey size on ln-transformed θ using ln-transformed SL as the covariate; prey size: $F_{2,22} = 26.58$, $P < 0.0001$).

Visual angle is assumed to be independent of prey size and to provide a measure of either the visibility of the prey or the visual capability of the predator. Prey size and the changing visual capabilities of fishes during growth play significant roles in estimation of the encounter probabilities required for most diet choice and habitat switching models where the availability of prey in the environment is a function of visibility (Werner and Hall 1974; O'Brien et al. 1976; Mittelbach 1981; Eggers 1982; Askes and Giske 1990, 1993). Because the distribution of attacks on prey differs significantly from that expected from prey visibility alone, planktivorous bluegill presumably incorporate a decision rule before they react to prey (Walton et al. 1992). Prey selection by small bluegill is constrained by several factors. In addition to locomotive and visual constraints, mouth gape and digestibility of prey are thought to constrain prey selection by small planktivorous fish (Mills et al. 1984; Walton et al. 1992). Our results indicate that visual angles of small bluegill are not independent of prey size. Consequently, when the composition of the preferred diet differs from the prey that are most readily detected, visual angle may not measure prey visibility or the fish's visual capability. This result draws into question the interpretation of reaction distance and visual angle studies, and their application to encounter rates, particularly for small fishes in nature. Prey size preferences and search behavior must be taken into account when using small fish and comparing behavioral measures of vision among species.

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Postfire responses of lotic ecosystems in Yellowstone National Park, U.S.A.

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Abstract: Wildfire is a major large-scale disturbance affecting terrestrial landscapes and lotic ecosystems in many regions of the world. We examined environmental and biological responses of 20 streams in Yellowstone National Park, U.S.A., over 5 years following extensive wildfires in 1988. Streams of burned catchments displayed increases in dissolved nitrate-nitrogen following the fires. Summer water temperatures often exceeded 20°C in small (first- and second-order) streams of burned catchments compared with <15°C in their unburned counterparts. Habitat heterogeneity decreased in streams of burned watersheds as demonstrated by changes in substrate embeddedness and near-bed velocities. Substantial alteration of channels and major restructuring and movement of large woody debris occurred in fire-impacted but not reference streams. Transported and benthic organic matter, mostly charcoal, increased in burned sites. No major changes were found in macroinvertebrate density, biomass, or richness, although significant changes occurred in relative abundances of miners, gatherers, and scrapers of burned sites. Chironomidae abundance was greater initially (postfire years 1–3), followed by later increases (postfire years 3–5) by the mayfly *Baetis bicaudatus* in burned sites compared with reference streams. Our findings demonstrate an integral relationship over time between a stream and its catchment, following large-scale disturbances such as wildfire.

Résumé : Les incendies de forêts constituent une grave perturbation à grande échelle pour les paysages terrestres et écosystèmes lotiques de nombreuses régions du monde. Par suite d'importants incendies de forêts qui ont sévi en 1988 dans le Parc national Yellowstone aux États-Unis, nous avons examiné sur 5 ans les réactions environnementales et biologiques de 20 cours d'eau. Nous avons constaté que les cours d'eau des bassins hydrographiques touchés par les feux présentaient des augmentations de l'azote et des nitrates dissous. De plus, la température estivale des petits cours d'eau (de premier et de second ordre) des bassins brûlés dépassait fréquemment 20°C, alors qu'elle est souvent <15°C dans les autres cours d'eau. Des changements dans la disposition du substrat et dans les vitesses du courant près du fond du lit reflètent une diminution de l'hétérogénéité des habitats dans les cours d'eau des bassins brûlés. Toutefois, des modifications importantes des chenaux ainsi qu'une restructuration et un déplacement de grande ampleur des gros débris de bois se sont produits dans les cours d'eau des régions brûlées, contrairement à ce qui s'est passé dans les cours d'eau de référence. La quantité de matière organique transportée et accumulée, essentiellement du charbon, a augmenté dans les sites brûlés. Nous n'avons constaté aucun changement dans la densité, la biomasse ou la richesse des macroinvertébrés, quoique d'importants changements se soient produits dans l'abondance relative des fouisseurs, des récolteurs et des déposivores des sites brûlés. Dans les trois premières années suivant le feu, les chironomidés étaient plus abondants dans les sites brûlés que dans les sites de référence; dans les deux années qui ont suivi, l'abondance de l'éphémère *Baetis bicaudatus* s'est accrue. Nos résultats démontrent l'existence d'un rapport temporel intégral entre un cours d'eau et son bassin hydrographique par suite d'une perturbation à grande échelle telle qu'un incendie de forêt.
[Traduit par la Rédaction]

Introduction

Globally, wildfire is one of the primary natural disturbances influencing the heterogeneity, patchiness, and diversity of terrestrial landscapes and the lotic ecosystems that drain these landscapes (White and Pickett 1985; Resh et al. 1988). Wildfire profoundly alters terrestrial systems and potentially sets the stage for future successional trajectories and ecosystem development (sensu Kay 1991). Wildfire management has important ramifications towards maintaining biodiversity of terrestrial and aquatic systems and the resulting stability and integrity of these ecosystems (Knight and Wallace 1989; Romme and Despain 1989). Thus, the paucity of information on the effects of wildfire on terrestrial and especially freshwater ecosystems is surprising (Minshall et al. 1989). Indeed, most papers examining fire effects on lotic systems have focused on changes in abiotic properties (flow, sediment, chemistry) (Tiedemann et al. 1979; Bayley et al.1992; Beaty 1994; Ewing 1996), with fewer studies addressing the response of biota (fish and macroinvertebrates) (Albin 1979; Richards and Minshall 1992; Minshall et al. 1995; Roby and Azuma 1995; Mihuc et al. 1996).

The effects of fire on stream ecosystems can be partitioned into (1) short-term (<1 year) changes arising directly from increased water temperatures and abrupt alterations in water chemistry and food quality and (2) delayed responses, including

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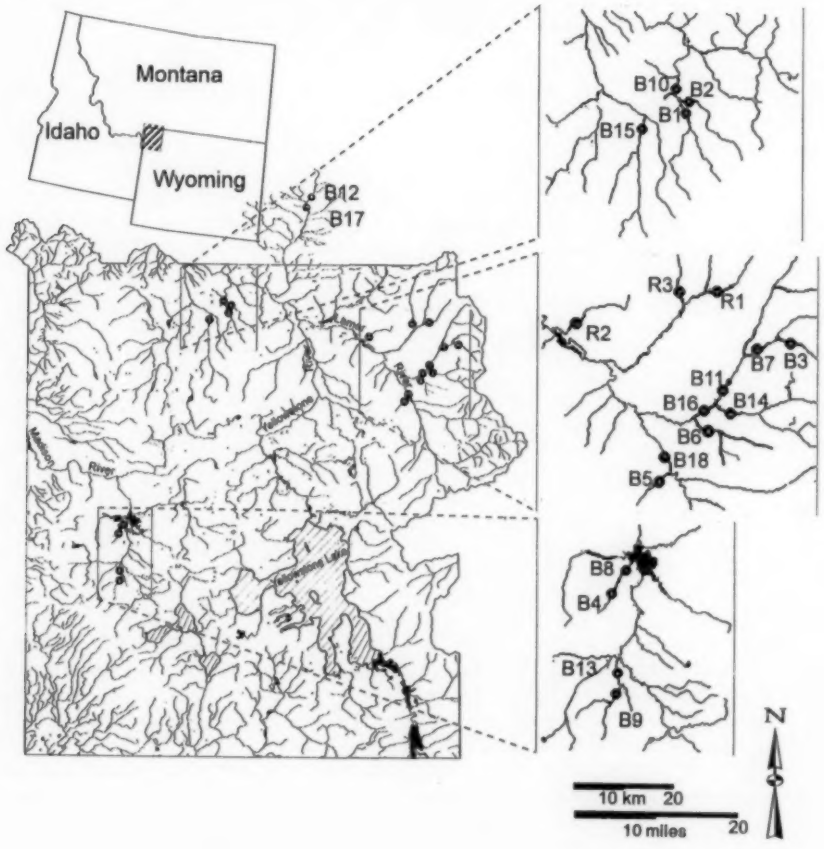
Fig. 1. Map of Yellowstone National Park and respective study sites. R, reference site; B, burned site. See Table 1 for stream number designations.



chemical, resulting from the removal and eventual successional replacement of the vegetative cover in stream catchments (Minshall et al. 1989; Minshall and Brock 1991). Delayed impacts can be separated further into midterm (~1–10 years) and long-term (>10 up to 100–300 years) effects. Our study addressed the short-term and midterm effects of fire on stream ecosystems following the conflagration of 1988 in Yellowstone National Park.

Most postfire effects are primarily attributed to physical disturbances associated with increased runoff and sediment scouring and are expected to exert their maximum influence within the first few years after fire, during the initial recovery of terrestrial vegetation. In addition, long-term changes in abiotic and biotic properties of lotic systems (i.e., alterations in food resources and retention capacity in fire impacted streams) are posited to reflect the subsequent recovery of riparian and upland terrestrial vegetative cover following wildfire (Molles 1982; Minshall et al. 1989). This study is one of the few systematic investigations of a landscape-level disturbance of stream ecosystems at a spatial scale of entire catchments and expected recurrence frequency of hundreds of years (Romme 1982; Reice et al. 1990). We focused on ecosystem properties that encompassed benthic macroinvertebrate communities, food resources (periphyton and benthic organic matter), water chemistry (including potentially limiting nutrients), and physical factors that would act as indices of stream ecosystem response to wildfire.

Description of study area and sites

The Greater Yellowstone Ecosystem has two distinct climates (Despain 1987): one type, commonly associated with the interior region and large valleys, has a spring peak in precipitation; the other, found on the western plateaus and the mountains of the northern and eastern parts, has a winter peak in precipitation. These two climates are reflected in the vegetation. Typical valleys are covered by dry grasslands and sagebrush (*Artemisia tridentata*) steppe communities. Douglas-fir (*Pseudotsuga menziesii*) is found on north-facing slopes (Despain 1987). In contrast, mountain areas usually are vegetated by conifer forests, especially Engelmann spruce (*Picea engelmannii*), or moist meadows. Most of our study sites were in areas of montane climate, but a few (Fairy Creek, Iron Springs Creek) were in the interior-type climate (Fig. 1). In Yellowstone, 75–85% of the precipitation occurs as snow or rainfall on snowpack; freezing air temperatures can occur in any month (Despain 1987).

We concentrated our efforts on 20 streams that ranged in

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Table 1. Coordinates and physical and chemical characteristics of streams examined in the study.

Stream	Map no.	Order	Coordinates	Catchment area (ha)	% catchment burned	Slope	Discharge (m^3/s)	Median substratum size (cm)	Near-bed velocity (cm/s)	Specific conductance (µS/cm)	pH	NO_3 (mg/L)	Ortho-P (mg/L)
Blacktail Deer, EF	B2	1	110°35″, 44°53″	1 281	92	2.0	0.031	7.5	0.15	126	8.4	<0.002	0.130
Blacktail Deer, WF	B1	1	110°35″, 44°53″	1 570	90	7.0	0.028	4.3	0.17	136	8.5	0.006	0.160
Fairy	B9	1	110°52″, 44°32″	428	76	1.0	0.054	0.1	0.25	66	8.1	0.008	<0.005
Twin	B5	1	110°10″, 44°48″	1 020	68	11.0	0.076	10.3	0.11	190	8.6	0.026	0.057
Upper Cache	B7	1	110°03″, 44°51″	138	64	10.0	0.001	5.3	0.04	153	8.0	0.230	0.180
Blacktail Deer, main	B10	2	110°35″, 44°53″	3 298	88	4.0	0.098	9.9	0.39	136	8.3	0.004	0.078
Fairy	B13	2	110°51″, 44°33″	1 199	81	0.5	0.092	0.4	0.07	318	9.3	<0.002	0.014
Upper Cache	B6	2	110°05″, 44°49″	377	71	10.0	0.004	4.7	0.11	132	8.2	0.008	0.150
Lower Cache	B3	2	110°05″, 44°50″	781	47	9.0	0.011	5.9	0.18	220	8.4	0.111	0.281
Iron Springs	B3	2	110°52″, 44°26″	615	41	14.0	0.065	3.3	0.22	48	7.8	0.019	0.013
Amphitheater	R1	2	110°04″, 44°56″	2 535	2	6.0	0.077	6.7	0.29	82	8.1	0.004	0.067
Rose	R2	2	110°13″, 44°54″	2 390	0	8.0	0.009	6.1	0.14	207	8.5	0.006	0.026
Cache	B11	3	110°04″, 44°51″	12 052	68	2.0	0.184	6.4	0.10	86	8.1	0.040	0.057
Hellroaring	B12	3	110°04″, 44°50″	3 975	59	3.0	0.101	7.7	0.12	85	8.2	0.002	0.041
Lava	B15	3	110°38″, 45°56″	8 968	47	2.0	0.622	30.5	0.44	72	7.8	0.041	0.022
Iron Springs	B4	3	110°38″, 45°56″	1 838	39	1.0	0.656	3.6	0.48	64	7.9	0.004	0.008
Cache, SF	B14	3	110°04″, 44°50″	5 781	39	3.0	0.174	5.5	0.13	105	7.9	0.004	0.095
Pebble	R3	3	110°07″, 44°56″	5 888	17	3.0	0.224	9.9	0.19	203	8.3	0.002	0.008
Cache	B16	4	110°05″, 44°50″	17 995	59	2.0	0.336	9.5	0.21	89	8.1	0.002	0.063
Lamar	B18	4	110°08″, 44°48″	NA	50	1.0	0.58 1	8.5	0.21	155	8.7	<0.002	0.069
Hellroaring	B17	4	110°23″, 45°09″	6 640	46	2.0	0.067	8.5	0.10	83	8.3	0.004	0.039

Note: Physical and chemical data recorded October 1988. Catchment size and percent catchment burned determined from GIS analysis. See Fig. 1 for map number. B, burned stream; R, reference stream.

size from first through fourth order (Table 1). Few catchments having streams larger than fourth order were affected directly by the fires in the Greater Yellowstone Ecosystem. The proportion of the catchment burned for each stream was estimated using GRASS 3.0, a Geographical Information System (GIS). However, about 10% of the burned areas were not registered using the GIS because understory burns are not detected by satellite imagery. Based on the GIS results, most sites had at least 40% of their catchment burned, although the range was from 0% at Amphitheater Creek to 92% for the east fork (EF) of Blacktail Deer Creek (Table 1). Smaller burned sites (first-order average = 78%) typically had a greater proportion of their catchment burned than larger streams (fourth-order average = 52%). Streams showing little of their catchment burned (e.g., <20%) were selected as reference sites.

Most small streams had higher gradients (usually >5% slope) than larger study streams (always <3% slope) with the exception of second-order Fairy Creek (Table 1). Discharge values reflected stream order and catchment area; upper Cache (first order) had the lowest flow (0.001 m^3/s) and Lamar the greatest flow (0.581 m^3/s). In general, low-gradient systems (e.g., Fairy and Iron Springs creeks) had smaller substrate particle sizes than high-gradient systems, although median substrata size was between 5.0 and 10.0 cm for most study streams. Exceptions included the Fairy Creek sites, with substrata consisting primarily of sand and pebbles, and Lava Creek, having mostly large particles (median size = 30.5 cm) of volcanic origin. Average near-bed velocities were lowest in first-order upper Cache (0.04 cm/s) and highest in third-order Iron Springs (0.48 cm/s). Most sites exhibited mean near-bed velocities between 0.10 and 0.20 cm/s (Table 1).

Methods

The sampling protocol focused on factors that would provide both a measure of the short-term impact (first 12 months) and a foundation for subsequent study of the mid- and long-term responses of stream ecosystems to wildfire. Consequently, most of our methods are routine in stream ecology and are described in detail in standard reference sources (Talling 1973; Weber 1973; Greeson et al. 1977; Lind 1979; Platts et al. 1983; Merritt and Cummins 1984; APHA 1989). Details for relatively nonroutine procedures completed each year are given below.

Five permanent transects were established at approximately 50-m intervals at each study site to measure channel morphology and riparian conditions over time. The location of the initial (monumented) transect at each site was predetermined from topographic maps and aerial reconnaissance. Annual temperature ranges (delta-T_{ann}) were obtained using minimum and maximum temperature recorders placed in each stream in September 1988. At sites where temperature recorders were lost between sampling occasions, delta-T_{ann} was estimated using the maximum water temperature at the time of sampling and the minimum temperature based on available evidence (e.g., ice cover). Most of the routine sampling occurred during baseflow conditions. Chemical measures recorded when sampling at each site included specific conductance (Orion model 250) and pH (Orion model 125). In addition, water samples were collected, stored on ice after respective preservation, and analyzed in the laboratory for alkalinity, total hardness, nitrate, and orthophosphorus using standard methods (APHA 1989).

Field maps of woody debris were drawn to scale for two 50-m reaches at each site beginning in 1988. All wood larger than 40 cm

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long or 2 cm in diameter was recorded each year. The number of pieces present each year was tallied from the debris maps and the amount of wood rearrangement summarized by counting the number of pieces lost or gained in each subsequent year. Substratum size (*y*-axis) and percent embeddedness were determined for 100 random observations distributed throughout a 200-m reach at each site (Leopold 1970; Platts et al. 1983). Embeddedness was visually estimated as the degree (nearest quartile percent) of interstitial filling of the substratum by fine particles (sand and silt). Water velocities (Ott C-1 current meter) near the stream bed and water depths also were recorded at these 100 random locations, although depths were not measured in 1990. Discharge also was calculated for each site at the time of sampling (Bovee and Milhous 1978).

Quantitative samples of periphyton were collected from rock substrata near each transect ($n = 5$ per site per sample date) (after Robinson and Minshall 1986) and analyzed for chlorophyll *a* and ash-free dry mass (AFDM) (Lorenzen 1966; Stockner and Armstrong 1971; APHA 1989). Transported organic matter (TOM) was collected using sets of nested-nets ($n = 2$ per site per sample date), separated into coarse particulate organic matter (CPOM, >1.0 mm) and fine particulate organic matter (FPOM, 0.52–1.0 mm) fractions, and quantified as AFDM. TOM was not collected at several third- and fourth-order sites in 1990 due to high runoff from a rainstorm. Benthic macroinvertebrates were sampled from one location near each transect ($n = 5$ per site per sample date) using a modified Surber sampler (mesh size = 250 μm) following procedures described in Platts et al. (1983). Benthic macroinvertebrates were hand-picked from each sample and identified to the lowest taxonomic unit feasible using a dissecting microscope at 10×. We evaluated among-year changes in density, biomass, species richness, and functional feeding groups (after Merritt and Cummins 1984) of macroinvertebrates. Benthic organic matter (BOM) collected in conjunction with the sampling of macroinvertebrates was quantified as AFDM. Prior to quantification, the percent charcoal (nearest quartile) of each TOM and BOM sample was estimated visually from dried samples spread on white paper.

Temporal change in stream habitat conditions was examined using summed among-year coefficients of variation (CVs) for important physical measures (e.g., see Robinson et al. 1994). Among-year CVs for these variables were derived from either annual averages (e.g., substrate size, water velocity, depth) or individual spot measures (i.e., chemical constituents). We used these data to test the hypothesis that streams experiencing major habitat changes over time would have greater CVs than streams showing less environmental change over time. Changes in the spatial heterogeneity of benthic habitats were assessed by comparing annual CVs for specific habitat measures (substrate size and embeddedness, near-bed velocities, water depth) among study years. Here, we posited CVs to either increase or decrease over time depending on the measure of interest. For summary purposes, streams were grouped by date and stream order with selected variables and then compared among dates for each stream order using ANOVA followed by Tukey's post hoc test (Zar 1984). When necessary, variables were transformed either $\log(x + 1)$ or arcsine(square root(x)) prior to statistical analysis to meet the assumption of normality (Zar 1984). Variables that still failed to meet the assumption of normality were analyzed using the nonparametric Kruskal–Wallis rank test followed by Dunn's mean comparison test (Zar 1984). Although this study encompassed a relatively large number of study streams, budgetary limitations restricted the number of reference streams to three, thus constraining some statistical inferences in the text. Further, readers are referred to Minshall and Robinson (1995) for additional data on individual stream response.

Results

Physical and chemical characteristics

Weather records for Yellowstone National Park are limited, and continuous flow records were available only for lower Blacktail Deer Creek. We present here the available prefire and postfire discharge records (U.S. Geological Survey; no data for 1946–1988) for Blacktail Deer Creek and the corresponding precipitation data (U.S. Weather Bureau) for the nearest recording station in Livingston, Mont. (Fig. 2). Winter–spring precipitation and total annual precipitation were similar between pre- and postfire years, although being slightly wetter before than after the fire. Baseflow and peak discharge also appeared comparable for the two data sets. In contrast, annual discharge was somewhat greater and the period of high flow associated with spring snowmelt runoff and its peak occurred earlier in the spring following the fires (Fig. 2).

Data collected in 1988 prior to any major runoff was used to characterize the initial chemical and physical conditions at a site. The water chemistry varied widely among study streams and reflected local geological conditions (Table 1). For example, second-order Fairy Creek showed higher specific conductance, pH, and alkalinity than all other sites as a result of geothermal inputs. Larger streams (fourth-order sites) exhibited less chemical variation than smaller sites, most likely due to the homogenizing effects of greater flow. Instream nitrate concentrations under baseflow conditions were <0.004 mg/L for most sites; notable exceptions included first-order upper and second-order Cache creeks. Instream phosphorus levels under baseflow conditions typically were <0.10 mg/L, although higher levels (>0.10 mg/L) were observed in the smaller Cache and Blacktail Deer creeks.

Although useful in characterizing the chemical conditions in the burned and reference streams, our data do not adequately reflect conditions during periods of runoff when elevated concentrations of some ions are expected. For example, pronounced pulses of nitrate and phosphate associated with snowmelt and precipitation were observed in several burned sites following the Yellowstone fires, including Cache and Blacktail Deer creeks (P.D. Sebesta, NASA Ames Research Center, Moffelt Field, Calif., personal communication). Concentrations at these times reportedly exceeded 0.5 mg/L for phosphate and 1–2 mg/L for nitrate and extended throughout the mid-June to mid-September 1990–1993 study period.

Maximum water temperatures were greater ($P < 0.01$) in first- and second-order burned sites than in reference streams and often surpassed tolerance levels of salmonids (i.e., 20°C) (Fig. 3). Average maximum water temperatures were >20°C in second-, third-, and fourth-order burned sites but <20°C in reference streams. Larger burned sites (i.e., third and fourth order) displayed average maximum temperatures comparable with the third-order reference stream, although third-order Cache Creek still attained maximum temperatures as high as 26°C. An exception to the above pattern was observed in the spring-fed Iron Springs Creek which maintained maximum water temperatures below 11°C for the second-order site and below 15°C for the third-order site during the postfire study years.

Water chemistry

Nitrate-nitrogen displayed over 20% more temporal change (measured as among-year CVs) in streams with burned catchments than in reference streams ($P < 0.11$), implying a strong influence of wildfire on instream nitrogen concentrations (Table 2). Robinson and Minshall (1996) found a direct relationship

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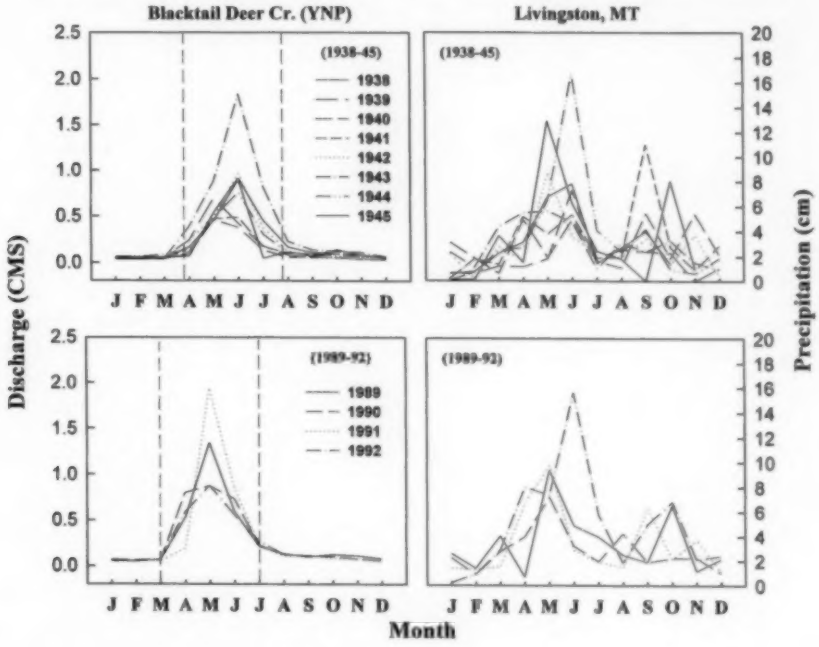
Fig. 2. Historical (1938–1945) and postfire (1989–1992) discharge and precipitation values. Note the change in timing of peak discharge in the postfire years.

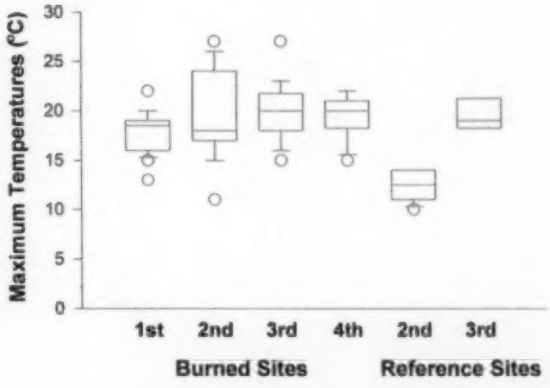


between loss of nitrogen and the percent catchment burned for these streams. Streams strongly influenced by extensive groundwater inputs (e.g., second-order Iron Springs) or that had highly confined channels due to geologic constraints (e.g., Lava Creek) typically had lower CVs for nitrate-nitrogen than other streams, regardless of the degree of catchment burned. Measures of ionic potential (i.e., pH, total hardness, total alkalinity, and specific conductance) exhibited similar and nonsignificant differences in mean CVs among stream groups ($P > 0.05$), indicating little temporal change in these constituents resulting from wildfire. Orthophosphorus had relatively high individual stream CVs; however, there were no significant differences in mean CVs among stream groups.

Woody debris

There was a substantial increase in the pieces of wood gained (attributed mostly to fire-felled trees) in most burned sites relative to reference streams (Fig. 4). Fewer pieces of woody debris were gained in larger (i.e., fourth order) than in smaller burned sites, with most of the increases in the number of wood pieces being observed in first- and significantly in third-order streams. For example, an average of 28 additional pieces of large woody debris per 50-m reach was recorded in third-order burned sites relative to only eight pieces gained in third-order reference streams in 1989 the first year following the wildfires. The source of wood in third- and fourth-order burned sites was equally partitioned between upstream (i.e., fire-felled trees) and riparian sources whereas wood in reference streams was primarily derived from the riparian zone (D.E. Lawrence, unpublished data). There was a significant loss of woody debris (>20 pieces/50 m) from burned sites in 1991 due to high discharge that was not evident in reference streams (<10 pieces/50 m)(Fig. 4). These data suggest that retention dynamics were altered (e.g., became less stable) in streams exposed to wildfire.

Fig. 3. Box plots of maximum water temperatures in different-sized burned and reference streams during the 5 years of study. Boxes represent means (central bar), standard deviations (box frame), 95% confidence limits (error bars), and outliers (circles) in maximum temperatures.



Channel morphology

The amount of channel cross-sectional area change over time was determined from the five permanent transects at each site and averaged by stream order. Relatively moderate change in channel morphology was observed for most burned sites between 1989 and 1990 (Fig. 5), probably due to below-normal

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Table 2. Average among-year CVs for measured water chemistry variables for streams, with burned catchments grouped by size and reference streams.

Stream	Total hardness	Total alkalinity	Specific conductance	Field pH	Ortho-P	Nitrate-N
Burned catchments						
First-order sites						
Blacktail Deer, EF	5	13	17	1	24	60
Blacktail Deer, WF	16	18	23	2	35	81
Upper Cache	21	12	10	3	27	54
Fairy	16	24	10	2	29	55
Twin	17	21	29	6	37	72
Mean	15.0	17.5	17.8	2.6	30.3	64.4
SD	5.2	4.6	7.3	1.8	4.8	10.6
Second-order sites						
Lower Cache	9	8	17	1	29	47
Upper Cache	26	18	14	1	13	55
Fairy	16	16	17	2	32	74
Iron Springs	17	10	20	1	44	35
Blacktail Deer, Main	5	10	32	5	31	72
Mean	14.6	12.4	20.0	2.0	29.8	56.6
SD	7.2	3.9	6.3	1.5	9.9	14.8
Third-order sites						
Cache	8	14	14	4	38	55
Hellroaring	13	6	28	1	30	122
Iron Springs	21	12	13	1	24	55
South Cache	16	12	21	6	23	70
Lava	11	15	19	1	23	27
Mean	13.8	11.8	19.0	2.6	27.6	65.8
SD	4.4	3.1	5.4	2.1	5.8	31.4
Fourth-order sites						
Cache	12	27	12	6	35	59
Hellroaring	6	6	24	2	20	69
Lamar	11	7	19	4	31	83
Mean	9.7	13.3	18.3	4.0	28.7	70.3
SD	2.6	9.7	4.9	1.6	6.3	9.8
Reference streams						
Amphitheater	10	19	17	1	5	48
Rose	21	14	27	1	33	31
Pebble	10	20	18	1	29	50
Mean	13.7	17.7	20.7	1.0	22.3	42.9
SD	5.2	2.6	4.5	0.0	12.4	8.5

spring runoff in 1989 resulting from low snow accumulation during winter (e.g., see Ewing 1996). Most changes observed in channel profiles in 1989 were from first- through third-order sites with steep gradients. For example, channel depth increased in Twin Creek and the west fork (WF) of Blacktail Deer Creek between 1988 and 1989 whereas low-gradient Fairy and third-order Iron Springs creeks showed little change in channel morphology between 1988 and 1990 (Minshall and Robinson 1995). These differences among streams of similar order but different gradients caused the high variability in the summarized data of burned sites relative to reference streams (Fig. 5). Major runoff events during 1991 and 1992 resulted in most burned sites experiencing significant channel alterations that were not observed either previously or in similarly sized reference streams. For example, the channel of third-order Cache Creek widened and shifted laterally in 1991 and 1992 whereas reference sites displayed no change in channel morphology throughout the study period (Minshall and Robinson 1995).

Small burned streams showed the least, although still substantial, average amount of channel change among burned sites because of lithological constraints (Fig. 5). For example, the survey reach on upper Cache Creek was predominantly on bedrock, thus prohibiting additional channel downcutting following the initial disturbance, and Lava Creek (a burned site) also displayed minimal morphological change because of its highly confined channel. Regardless, high-gradient first- and second-order sites, e.g., Cache, WF and main Blacktail Deer, and Twin creeks, still displayed major changes in channel morphology in 1991 and 1992 (Minshall and Robinson 1995). Larger streams showed temporal patterns similar to smaller burned sites, with little channel change occurring in the low-gradient systems (e.g., third-order Iron Springs Creek) or in the reference Pebble Creek.

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Fig. 4. Mean (+1 SD) number of pieces of wood gained or lost per 50 m counted in burned and reference streams by stream order from 1989 through 1992. Asterisks indicate sites that displayed significant differences ($P < 0.05$) among years.

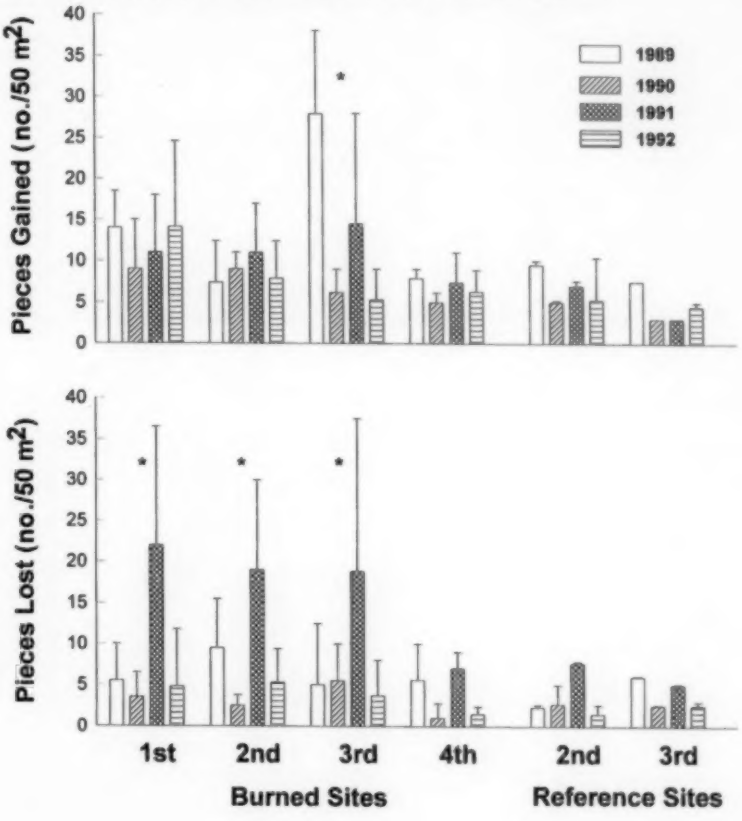

Fig. 5. Mean (+1 SD) change in channel area for burned and reference streams by stream order for October 1988 and August 1989–1992. Variation among individual burned sites is reflected in the large standard deviations. The asterisk indicates significant differences ($P < 0.05$) among years within the stream size.

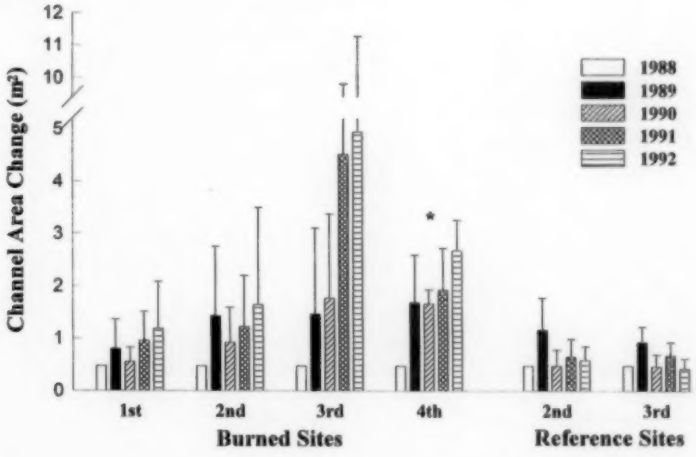

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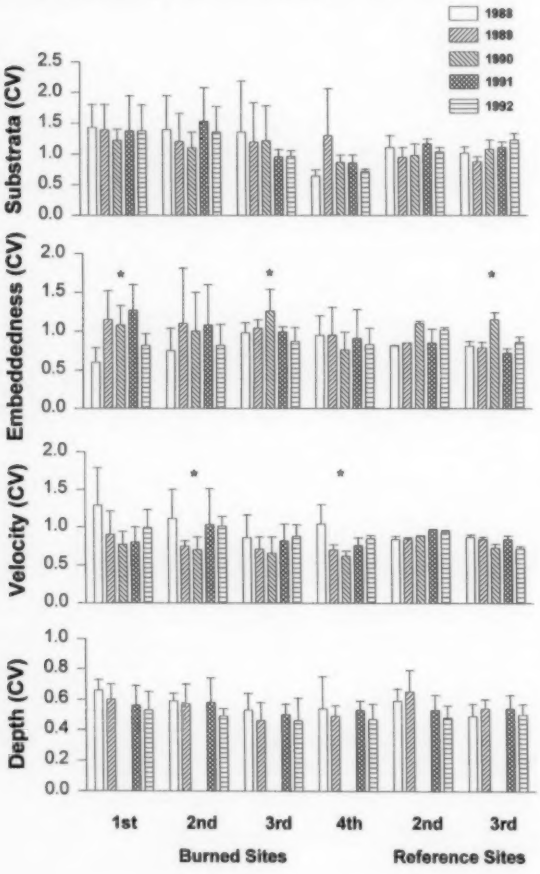
Fig. 6. Mean (+1 SD) annual CVs for stream substrata size, substrata embeddedness, near-bed water velocity, and water depth. See text for sample sizes and method of determination. Asterisks indicate sites with significant differences ($P < 0.05$) among years.



Instream habitat heterogeneity

Temporal changes in instream habitat conditions were assessed using CVs from the annual measures of substratum size and embeddedness, near-bed velocity, and water depth. There were no significant differences among substrata CVs over time for burned or reference streams (Fig. 6), suggesting little change in substrate heterogeneity resulting from the fires. In contrast, substrate embeddedness CVs significantly increased in first-order burned sites (with a similar trend in second-order sites) in postfire years 2–4, which was not seen in larger burned or reference streams. The differences in substrate embeddedness CVs in small burned sites indicate subtle changes in the amount and movement of fine sediments in these streams over time. For example, Minshall and Robinson (1995) suggested that a pulse of fine sediments had moved from smaller burned sites into larger streams during the first few postfire years. CVs for near-bed water velocity decreased in most burned sites after 1988 ($P < 0.10$), but were similar between years in reference streams ($P = 0.67$), indicating a reduction in the heterogeneity of near-bed water flow in the burned sites since the fire (Fig. 6). There were no significant differences among annual CVs for water depth in burned or reference streams.

Environmental conditions over time

To evaluate whether streams of burned catchments experienced any increased physical change over time relative to reference streams, we calculated among-year CVs for a number of important habitat variables and then summed these CVs for each study site (Table 3). The summed CVs were 100–200% higher in first- to third-order burned sites than in reference streams ($P < 0.001$) whereas fourth-order burned sites displayed values similar to those of reference sites ($P = 0.05$). Further, summed habitat CVs showed a strong positive regression against the percent of each catchment burned ($r^2 = 0.67$, $P = 0.05$), except for WF Blacktail Deer, inferring a strong physical response of stream ecosystems to wildfire. In addition, average summed CVs decreased as stream size increased, suggesting less influence of fire on the physical conditions of larger stream ecosystems. The exception, WF Blacktail Deer Creek, displayed low CVs for variables derived from changes in channel morphology.

Biological relationships

TOM and BOM

TOM, as CPOM or FPOM, generally increased in all burned sites and consisted primarily (>50%) of burned material (Fig. 7). High CPOM and FPOM levels after 1988 for burned sites relative to reference streams probably reflect increased overland input in burn catchments from increased runoff. The percent charcoal decreased and amorphous detritus increased in TOM in all burned sites after 1990 ($P < 0.05$), suggesting that much of the transported matter in 1990 was derived from new riparian growth or autotrophic production. However, the percent charcoal of TOM increased again in 1991 in burned sites, indicating substantial overland input of burned organic matter resulting from major runoff events during that year. The percent charcoal of TOM was always low in samples from reference streams.

The amount of benthic organic matter increased in most burned sites the first year following the fires and then decreased to reference levels by 1991 ($P < 0.05$) (Fig. 8). BOM levels were similar among years in respective fourth-order burn and reference sites ($P = 0.05$). About 20–40% of the benthic organic matter in burned sites was of burned material whereas the percent charcoal of BOM in reference sites remained below 20% throughout the study ($P < 0.01$). Mean periphyton standing crops (as chlorophyll *a* and AFDM) were greatest in October 1988 (two to five times) ($P = 0.05$), decreased in summer 1989, and then increased in subsequent summers in most burned and reference streams (Fig. 8).

Benthic macroinvertebrates

The average density of macroinvertebrates was substantially lower in third- and significantly lower in fourth-order burned sites in 1989 than in other years whereas no change occurred among years in reference streams ($P > 0.27$) (Fig. 9). The average biomass of macroinvertebrates increased, albeit nonsignificantly, from 1988 through 1990 and then decreased to 1988 levels by 1991 in burned sites; this trend also was not found in reference streams. There were no significant changes in mean

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Table 3. CVs for physical measures recorded annually at each site during the 5-year study.

Stream	Order	Annual temperature range	Channel area change	Ratio channel area	Width/ depth ratio	Bank full channel width	% embeddedness	Bottom velocity	Water depth	Substrate size mean	Substrate size median	Summed CVs
Burned catchments												
First-order sites												
Blacktail Deer, EF	1	0.09	0.47	0.10	0.70	0.67	0.41	0.30	0.12	0.42	0.32	3.61
Blacktail Deer, WF	1	0.10	0.21	0.09	0.18	0.07	0.31	0.25	0.16	0.14	0.29	1.79
Upper Cache	1	0.06	0.62	0.03	0.64	0.49	0.32	0.38	0.34	0.27	0.49	3.64
Fairy	1	0.02	0.49	0.19	0.59	0.54	0.26	0.40	0.11	0.19	1.00	3.81
Twin	1	0.07	0.54	0.04	0.30	0.30	0.33	0.62	0.17	0.13	0.22	2.74
Mean		0.07	0.47	0.09	0.48	0.41	0.33	0.39	0.18	0.23	0.46	3.12
SD		0.03	0.15	0.06	0.23	0.23	0.05	0.14	0.09	0.12	0.32	0.85
Second-order sites												
Lower Cache	2	0.03	0.61	0.04	0.28	0.56	0.09	0.24	0.35	0.24	0.07	2.52
Upper Cache	2	0.17	0.69	0.03	0.59	0.56	0.19	0.29	0.07	0.15	0.50	3.24
Fairy	2	0.06	0.37	0.06	0.57	0.07	0.43	0.45	0.49	0.72	0.82	4.04
Iron Springs	2	0.21	0.36	0.19	0.41	0.20	0.28	0.29	0.31	0.87	0.24	3.35
Blacktail Deer, main	2	0.07	0.90	0.15	0.26	0.49	0.24	0.27	0.24	0.40	0.11	3.13
Mean		0.11	0.59	0.10	0.42	0.38	0.25	0.31	0.29	0.47	0.35	3.26
SD		0.08	0.23	0.07	0.15	0.23	0.12	0.08	0.15	0.31	0.31	0.54
Third-order sites												
Cache	3	0.09	0.77	0.09	0.23	0.12	0.21	0.94	0.11	0.28	0.37	3.21
Hellroaring	3	0.15	0.42	0.05	0.20	0.06	0.40	0.34	0.15	0.11	0.15	2.04
Iron Springs	3	0.15	0.30	0.16	0.27	0.30	0.22	0.52	0.08	0.47	0.21	2.68
South Cache	3	0.20	0.54	0.06	0.21	0.17	0.11	0.31	0.09	0.25	0.27	2.21
Lava	3	0.09	0.85	0.18	0.09	0.02	0.17	0.41	0.08	0.72	0.60	3.21
Mean		0.14	0.58	0.11	0.20	0.14	0.22	0.50	0.10	0.37	0.32	2.67
SD		0.04	0.23	0.06	0.07	0.11	0.11	0.26	0.03	0.24	0.18	0.55
Fourth-order sites												
Cache	4	0.13	0.48	0.03	0.30	0.03	0.26	0.22	0.21	0.10	0.28	2.05
Hellroaring	4	0.13	0.42	0.05	0.20	0.06	0.40	0.34	0.15	0.11	0.15	2.03
Lamar	4	0.07	0.49	0.01	0.08	0.03	0.19	0.37	0.07	0.13	0.30	1.75
Mean		0.11	0.46	0.03	0.19	0.04	0.28	0.31	0.14	0.11	0.24	1.94
SD		0.04	0.04	0.02	0.11	0.02	0.11	0.08	0.07	0.01	0.08	0.17
Reference streams												
Amphitheater	2	0.13	0.45	0.07	0.21	0.23	0.13	0.06	0.17	0.12	0.27	1.83
Rose	2	0.10	0.38	0.02	0.09	0.04	0.15	0.24	0.10	0.13	0.15	1.41
Pebble	3	0.09	0.32	0.03	0.15	0.02	0.21	0.22	0.14	0.20	0.32	1.70
Mean		0.10	0.38	0.04	0.15	0.10	0.16	0.17	0.14	0.15	0.25	1.65
SD		0.02	0.07	0.03	0.06	0.11	0.04	0.10	0.03	0.04	0.08	0.21

taxonomic richness or Ephemeroptera–Plecoptera–Trichoptera (EPT) taxonomic richness among sample years in burned or reference streams ($P > 0.05$) (Fig. 9). Minshall and Robinson (1995), however, documented a significantly reduced taxonomic richness (~10 taxa) in March 1989 (prior to the first spring snowmelt runoff after the fires) in some of the smaller burned sites that was not evident in larger burned sites or reference streams. This decrease in species richness suggests a more immediate effect of the fires on the macroinvertebrate communities in smaller streams, perhaps related to a reduced quality of food resources.

All burned sites showed major increases in the relative abundance of miners the first 2 years following the wildfires ($P < 0.10$) that were not evident in reference streams (Fig. 10). For example, miners constituted >50% of the assemblage in burned sites but <30% in reference streams in these years. Miner abundance decreased in burned sites to reference levels by 1991 (postfire year 3), being replaced with an increase in the relative abundance of gatherers ($P < 0.10$). In general, the abundance of scrapers was substantially less in first- and second-order burned sites than in third- and fourth-order burned and reference streams. In fact, scraper abundance actually increased in fourth-order burned sites during postfire years 2 and 3 ($P = 0.05$). Shredders increased in relative abundance only in the first-order burned sites in postfire years 3 and 4. The relative abundance of predators and filterers remained unchanged in burned and reference streams following the fires ($P > 0.05$).

Of the most abundant taxa, chironomids increased in postfire years 1 and 2 followed by a decrease in subsequent study

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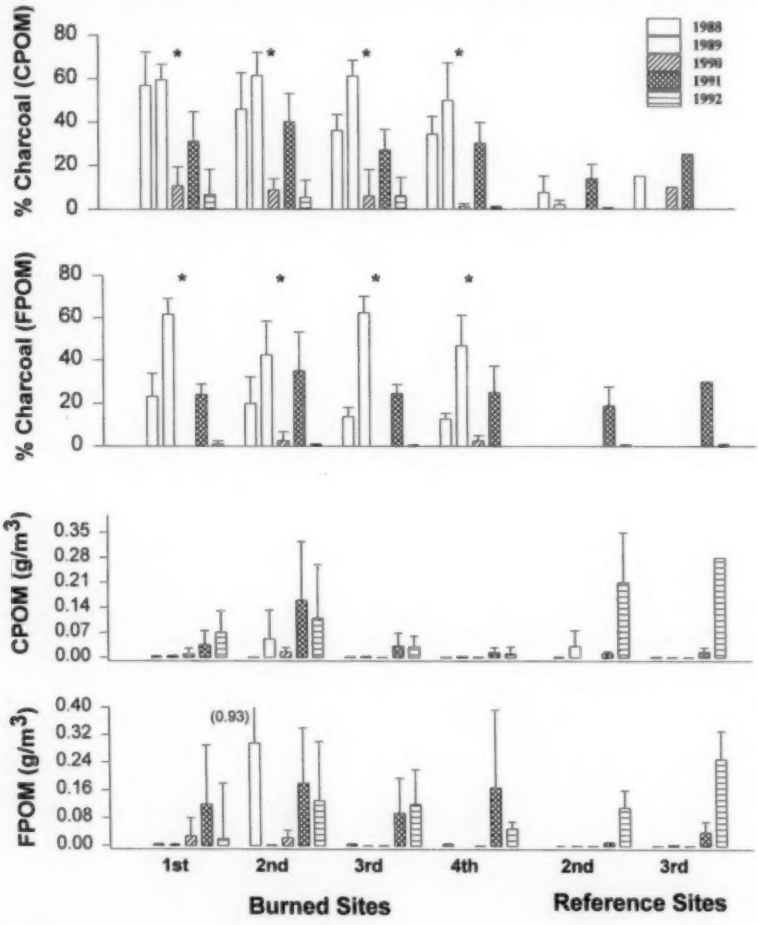
Fig. 7. Mean (+1 SD) TOM as CPOM or FPOM and percent charcoal of CPOM and FPOM from burned and reference streams by stream order from 1988 through 1992. See text for sample sizes. Asterisks indicate sites with significant differences ($P < 0.05$) among years.



years in all burned sites ($P = 0.05$) (Fig. 11). Indeed, chironomids constituted >40% of the assemblage in most burned sites but <30% in reference streams in the first two postfire years. *Baetis bicaudatus* increased in abundance, especially in third- ($P < 0.06$) and fourth-order ($P < 0.01$) sites, in burned sites each year following the fires and represented >20% of the macroinvertebrate assemblage in these streams by 1992. The abundances of chironomids ($P > 0.99$) and *Baetis* ($P > 0.59$) remained essentially unchanged among years in reference streams. The Oligochaeta displayed similar trends as *Baetis*. *Drunella doddsi* increased in abundance, constituting about 15% of the assemblage, in third- and fourth-order burned sites in postfire years 3 and 4 ($P = 0.05$).

In contrast, *Cinygmula* abundance decreased over time in burned sites ($P < 0.05$) and displayed a similar trend in reference streams ($P < 0.02$) (Fig. 11). *Ephemerella infrequens* became more abundant, although nonsignificantly, in burned sites and also increased in relative abundance in the third-order reference stream. *Rhithrogena* densities were extremely reduced in first- and second-order burned sites relative to second-order reference streams, but increased in abundance to >14% of the assemblage in third- and fourth-order burned sites by postfire year 2 ($P < 0.05$). *Suwallia* abundance was greater in 1988 ($P = 0.05$) than in subsequent years in small burned sites; this was not observed in reference streams. *Zapada columbiana* maintained similar abundances among years in burned and reference streams ($P > 0.05$).

Discussion

Immediate effects

Our knowledge of the immediate effects of the Yellowstone fires is based on measurements and direct observations of the study streams and their catchments during the first several weeks following burning. Losses in catchment and riparian vegetation and almost instantaneous conversion of terrestrial vegetation to charcoal and ash were obvious and expected. These resulted in immediate changes in the amount of light and quality of materials, e.g., allochthonous food resources, entering the streams. Although most burned trees remained standing, many downed trees and large limbs were observed bridging and in streams. The most striking immediate

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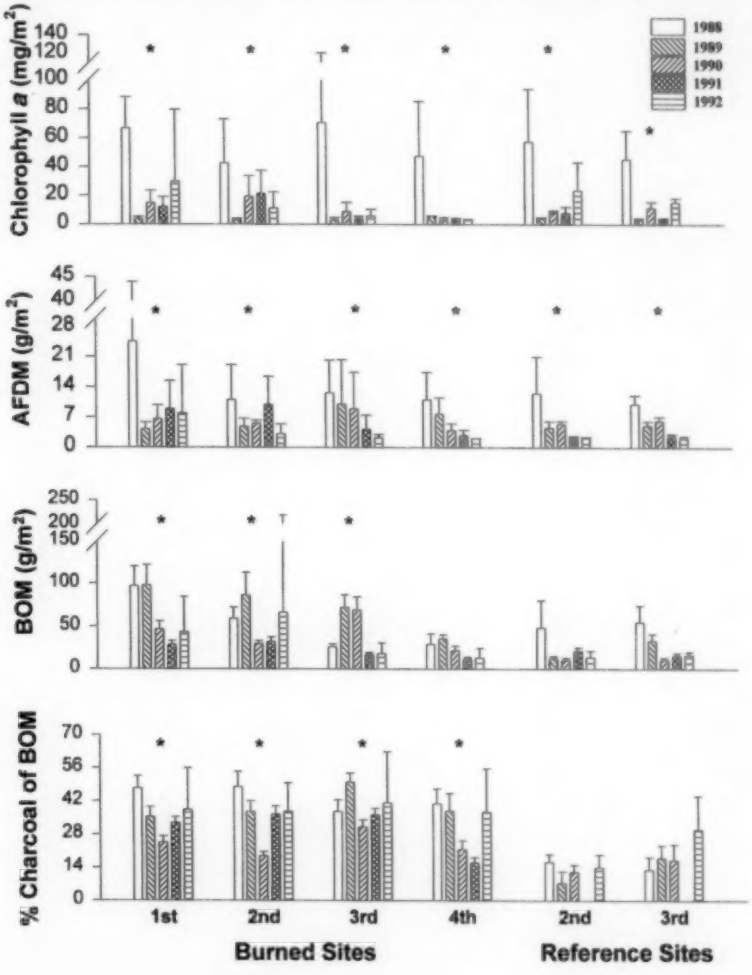
Fig. 8. Mean (+1 SD) periphyton chlorophyll *a* and AFDM, BOM, and percent charcoal of BOM for the burned and reference streams by stream order from 1988 through 1992. See text for sample sizes. Asterisks indicate sites with significant differences ($P < 0.05$) among years.



changes within the stream channel were the incineration and scorching of emergent mosses and heat fracturing (splaying) of rocks in and adjacent to first- and second-order streams. We also counted up to 10 dead cutthroat trout (*Oncorhynchus clarki*) per 250 m in third-order Cache and WF Blacktail Deer creeks.

Minshall et al. (1990) found that most dissolved chemical measures increased in streams of burned catchments the first year following the fires. Spencer and Hauer (1991) observed dramatic and rapid increases in phosphorus and nitrogen levels in streams during fire and attributed these increases to ash and to smoke gases, respectively. We suggest that high ammonia levels, highly soluble in gaseous form, arising from the smoke probably were responsible for the fish mortalities. Little or no immediate deleterious effect of fire was evident in periphyton standing crops or macroinvertebrate assemblages, even in the smallest streams observed (also see Albin 1979). These impacts are more difficult to discern due to the small size and rapid decay rates of the organisms involved. However, lotic macroinvertebrates are known to be adversely affected by exposure to ammonia (Gammeter and Frutiger 1990). Roby and Azuma (1995) showed that macroinvertebrate abundance and diversity were greatly reduced 3 weeks following wildfire in a northern California stream.

As hypothesized by Minshall et al.(1989), this study revealed distinct differences in the effects of wildfire on streams of different size. Following fire, headwater streams (first and second order) were more physically and chemically variable than intermediate-size burned streams (third and fourth order) or reference streams. In general, smaller streams had a greater proportion of their catchments burned than larger streams. For our study streams, the mean catchment burned was 75% for first- and second-order streams and 50% for third- and fourth-order streams. However, we also observed during aerial and ground reconnaissance that the catchments of many fire-impacted third- and fourth-order streams throughout Yellowstone Park and along its northern boundary with Montana were <50% burned and that this amount was much less for even

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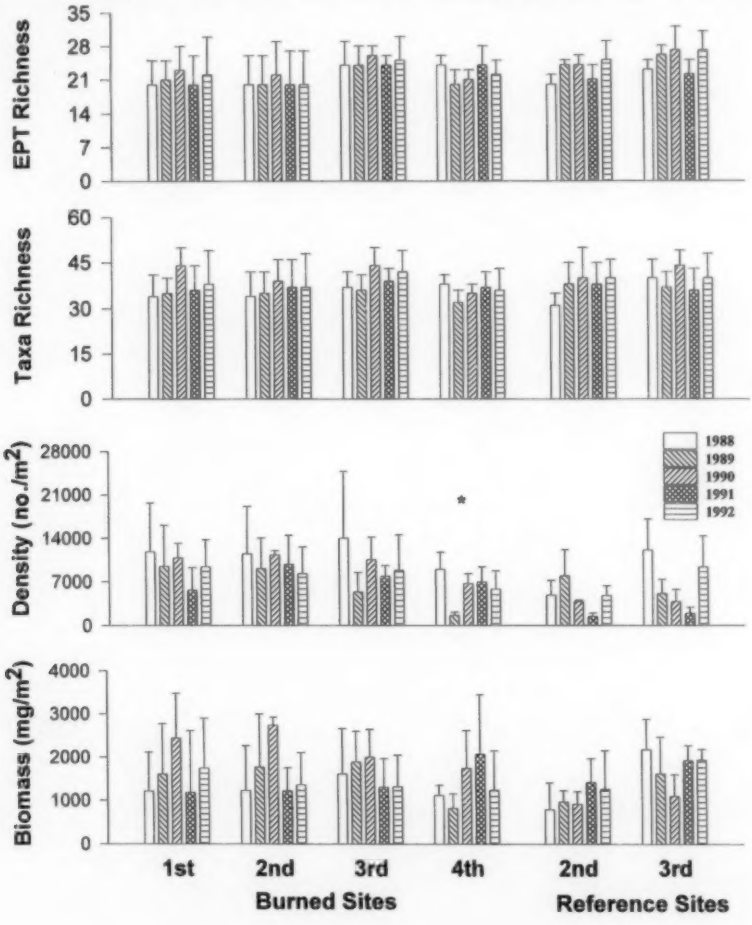
Fig. 9. Mean (+1 SD) macroinvertebrate EPT richness, taxa richness, density, and biomass in burned and reference streams by stream order from 1988 through 1992. See text for sample sizes. The asterisk indicates a site showing significant differences ($P < 0.05$) among years.



larger streams (no streams larger than sixth order are found in the park). As a result, the impact on biological properties also appeared more pronounced in smaller streams, although intermediate-size burned streams located in steep terrain with confined flood plains (e.g., third-order Cache and Hellroaring creeks) experienced greater amounts of overland flow and associated effects on the biota than other large study streams (Minshall and Robinson 1995).

The most consistent outliers from the general patterns found in this study were Fairy and Iron Springs creeks and were attributable to one or more relatively unique features. For example, besides being located in a different climate and underlain by different base rock (rhyolite) than other streams, (1) Fairy Creek sites had the lowest gradients of any study stream and the second-order site was unforested and strongly influenced by geothermal springs and (2) a large proportion of flow in Iron Springs Creek was groundwater; consequently, the third-order site displayed little variation in flow and usually did not freeze over in the winter.

Short-term effects

Minshall et al. (1989) showed that from October 1988 to March 1989, macroinvertebrate abundance, richness, and H' diversity decreased in six of eight monitored burned sites whereas these values increased or remained constant in the reference streams. Because no physical disturbances from runoff occurred during this period, we attribute these changes to high amounts of charcoal in stream benthos (>40%) and transport as a result of the fires. We further suggest that the input of charcoal decreased the palatability and quality (e.g., increased C:N values) of the organic matter pool as food. For example, Mihuc and Minshall (1995) found that only one taxon of 11 examined could utilize burned organic matter as a food source. Further, periphyton biomass decreased in burned streams (except third-order Iron Springs) during this period, although comparable changes were observed in reference streams. Data since 1989 indicate that burned organic matter is still being added to burned streams but in reduced amounts. Britton (1990) showed that BOM quantity and quality were altered the

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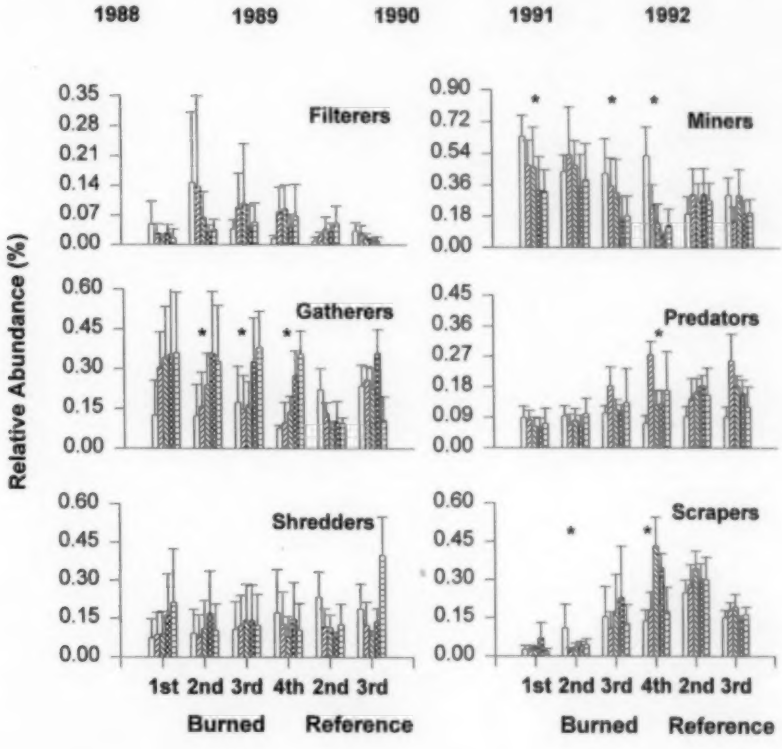
Fig. 10. Mean (+1 SD) relative abundances of macroinvertebrate functional feeding groups (after Merritt and Cummins 1984) for burned and reference streams by stream order from 1988 through 1992. See text for sample sizes. Asterisks indicate sites showing significant differences ($P < 0.05$) among years.



first year following a prescribed burn but that the effect was short-term due to fast recovery by riparian vegetation. After 1990 (postfire year 2), most fire-related effects appear to be caused by physical disturbance of the stream bed associated with high runoff rather than changes in food resources.

Spring melting of the 1989 snowpack was much slower than anticipated (P. Farnes, SCS, Bozeman, Mont., personal communication). Consequently, although several periods of "blackwater" associated with overland flow from heavy rains occurred between runoff and our August sampling, stream bed erosion and channel alterations were generally much less than expected or than occurred in later years. However, several first- through third-order streams, particularly Cache Creek and Hellroaring Creek catchments, did show substantial channel alteration and rearrangement of woody debris (Lawrence 1991). In addition, reductions in flow and substrate heterogeneity were observed in the burned streams, as indicated by changes in annual CVs in these measures, between 1988 and 1990. No comparable changes in either velocity or substratum occurred in our reference streams. A number of studies have documented similar changes in burned streams resulting from increased sediment loads and peaks in runoff (Tiedemann et al. 1979; Kusaka et al. 1983; Mitsudera et al. 1984; Helvey et al. 1985; Beaty 1994). Specifically, Beaty (1994) documented that median particle size initially decreased and then increased over time in a small stream following fire.

As documented in other studies, most dissolved constituents, especially nitrates, were higher in August 1989 than in October 1988, apparently in response to rainstorms during or immediately prior to the summer 1989 sample collections (also see Tiedemann et al. 1978; Nakane et al. 1983; Davis 1989; Britton 1991). In contrast with other ions (e.g., phosphorus, Spencer and Hauer 1991) that displayed only immediate changes in concentrations, temporal changes in instream nitrate levels typically reflected recovery by adjacent terrestrial vegetation (Bayley et al. 1992). Similarily, Robinson and Minshall (1996) found a direct correlation between nitrate loss and percent catchment burned in the Yellowstone study streams. Further, temporal changes in the quantity and quality of TOM and BOM between 1988 and 1989 suggest that charcoal and fine sediment were being transported from headwater streams and deposited downstream. Lastly, stream temperatures increased in smaller burn streams but remained unchanged in reference streams (also see Cushing and Olson 1963; Albin 1979).

Midterm effects

Although some major effects of fire were evident in the first three postfire years, burned streams appeared to be on a "fast recovery track" (sensu Minshall and Brock 1991). However, postfire year 3 (1991) was marked by at least two large runoff events that caused major physical changes in streams of burned watersheds with moderate to steep gradients. Ewing (1996) also noted that suspended sediment loads in the Lamar River

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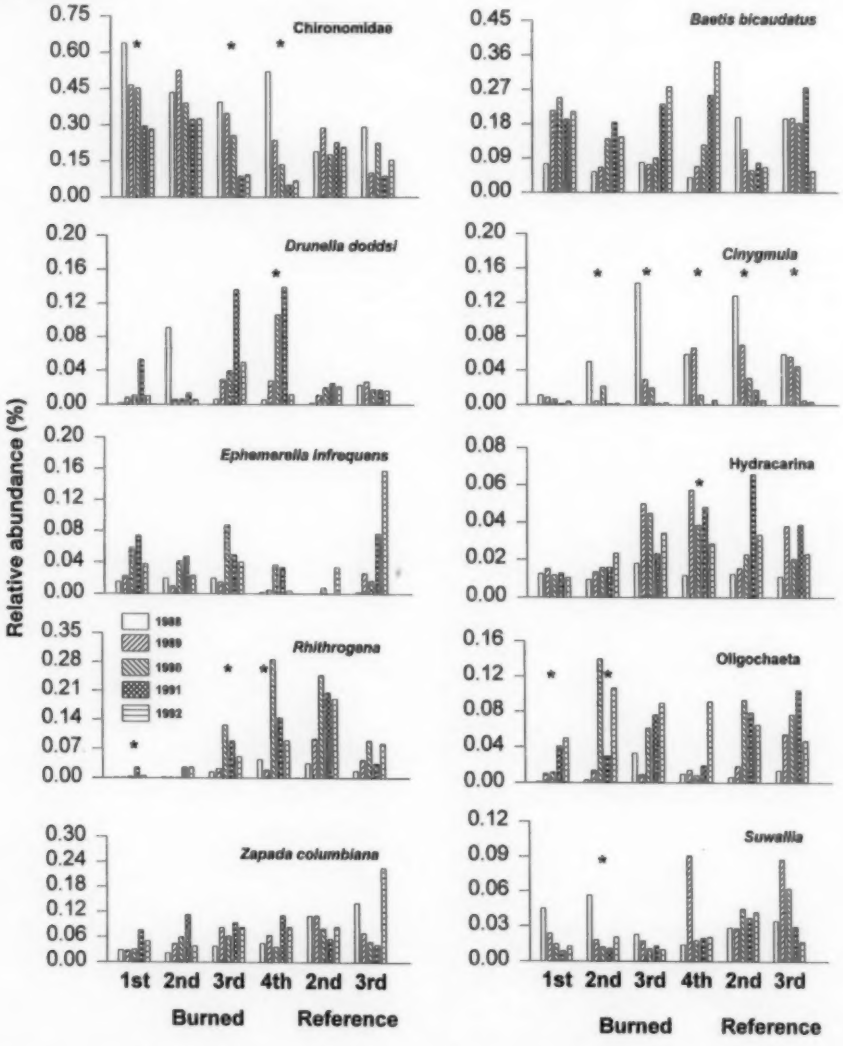
Fig. 11. Mean relative abundance of the 10 most common macroinvertebrate taxa collected in burned and reference streams by stream order from 1988 through 1992. Error bars deleted for clarity of presentation. See text for sample sizes. Asterisks indicate sites showing significant differences ($P < 0.05$) among years.



were much higher in 1991 and reflected the greater precipitation in this year. Additional channel modifications were observed in 1992, especially in the Cache Creek catchment. These disturbances were reflected in declines in biotic properties and served as important "resets" or delays in lotic ecosystem recovery (Minshall et al. 1989; Minshall and Brock 1991). Long-term studies are required to determine whether the frequency and magnitude of such disturbances will decrease over time as vegetation on the surrounding landscape recovers (sensu Minshall et al. 1989).

Our results show the importance of stream discharge and gradient in mediating physical disturbances associated with adverse intermediate effects (e.g., channel scouring and sediment loading) resulting from wildfire. High-gradient streams responded sooner (i.e., at lower flows) than low-gradient streams. For example, high-gradient tributaries showed major bed scouring and channel alteration the first two postfire years. At comparable discharges, high-gradient streams undergo greater physical disturbance than low-gradient streams. For instance, high-gradient burned streams displayed major changes (cutting or filling) in channel cross-section morphology in 1991 and 1992 whereas channel morphology of low-gradient burned streams and reference streams remained relatively constant.

Embeddedness data suggest that a pulse of fine sediments moved from burned watersheds into headwater streams and then gradually into larger burn streams over time. Median substrate size also decreased in first- through third-order burn streams following 1988 and remained low through 1992 (G.W. Minshall, unpublished data; also see Beaty 1994). An unexpected finding from our study was the maintenance of large amounts of fine inorganic sediments in headwater burn

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streams. From our study of streams in central Idaho (Minshall et al. 1990), we expected these materials to be rapidly removed and then increase again after 5–10 years. Because Yellowstone streams have lost a considerable amount of retentive capacity (Lawrence 1991; McIntyre and Minshall 1996) and periodically scour during high discharge, we believe that the "maintenance" of silt and sand is due to continued input from the surrounding catchment. This continued input also is suggested by the increase in percent charcoal of BOM in 1992. Comparable off-stream storage of fine materials apparently does not occur in the steeper, rockier central Idaho streams.

Woody debris in streams stabilizes stream channels (Nakamura and Swanson 1993), retains organic matter and sediment (Smith et al. 1993*a*, 1993*b*), and provides habitat important for fish and macroinvertebrates (Bilby and Likens 1980). First- through third-order streams contained the most wood and can be attributed to the lower competency of high flows to move larger pieces of wood and the closer proximity of trees to the main channel in smaller streams. In larger streams, high flows moved even the largest pieces of wood (including whole trees), leaving few pieces to stabilize the low-flow channel for more than a year. Small streams had lower debris volumes because a large proportion of fallen trees remained outside the channel margin. Bilby and Likens (1980) found an inverse relationship between stored organic matter and stream size, where first-order streams contained 75% and third-order streams held only 20% of the organic matter in the stream channel. Our data suggest that this relationship is altered in streams influenced by wildfire because of the reduced retention capacity in small burned streams (Lawrence 1991; McIntyre and Minshall 1996).

The effect of riparian zone burning was greatest in smaller streams and, as seen in this study, relatively fewer pieces of wood fell into the larger channels. Fallen wood in large streams is almost all drift or ramps (D.E. Lawrence, unpublished data), with few trees being tall and large enough to cross the entire channel. Wood in larger (third- and fourth-order) streams are found mostly outside the low-flow boundary because either a wood piece is outside the high-flow margin and thus stable or high flows deposit wood as drift onto braided gravel bars and high-flow point bars. Few pieces of wood are found in low-flow channels of larger streams, and thus, woody debris exerts little control on stream morphology. Robison and Beschta (1990) found increases in wood volume with increasing size of Alaskan streams, but they studied low-gradient streams where larger pieces of wood are stabilized for longer periods of time. In our study, large streams apparently are steep enough to move entire trees (75 cm diameter at breast height, >20 m long) having root wads.

Even 2 years following the 1988 fires, greater gross change in woody debris was present in burned than in reference streams due to an escalated gain in wood pieces. These newly fallen pieces are not secured and move more often than anchored wood. Bilby (1984) found that rearrangement of wood in streams of logged catchments was greater than in streams of unlogged catchments; a similar pattern was found in the present study with respect to fire. Associated with greater wood movement is the degree of channel scouring; disturbed streams experienced over 75 cm of scour in both studies. In contrast with logging impacts, we not only found an increase in woody debris loading immediately following catchment fire, but large snags will be available to enter the channel for about the next 20 years (Lyon 1984). Although significant rearrangement of woody debris in channels is still taking place, much woody debris is available to stabilize burn streams once side-slopes recover. For example, woody debris loads in burned sites were higher than in reference streams in 1992 (postfire year 4), but gains and losses of wood were comparable between the two stream types.

Preliminary predictions for stream habitat development can be made for streams in burned catchments based on our short- and mid-term results. Nearly all streams, especially second-order streams, are accruing pieces of wood. Keller and Tally (1979) found that between 50 and 100% of pool habitats in second- to fourth-order streams were formed by debris dams and more pools were debris related with increasing stream slope. As wood stabilizes, longer lasting pools are expected to form that will increase habitat for fish (Minshall et al. 1990; Nakamura and Swanson 1993). Because more wood was found in burned sites, we anticipate that more pools will form than in corresponding reference streams. In turn, an increase in adult fish density should accompany habitat development for up to 50 years following catchment fires. After that time, log decomposition and lack of large replacement trees may cause decreases in pool size and frequency and thus reduce fish habitat. Large trees will again enter stream channels forming deep pools and maximizing fish habitat around 150 years following fire, as found in Oregon streams (G. Reeves, U.S. Forest Service, Corvallis, Oreg., personal communication). However, habitat diversity decreased in Oregon streams of climax forests and this suggests that wildfire may play an integral role in creating more optimal habitat for fishes.

Macroinvertebrate communities in burned sites displayed major changes in response to the observed changes in the physical environments. For example, burned sites exhibited functional feeding group composition different from that in reference streams, suggesting alterations in food resources and a shift to more trophic generalists (e.g., Mihuc and Minshall 1995). However, the macroinvertebrate responses appeared to be more individualistic rather than associated with community properties. Community properties, such as species richness and diversity, showed substantial recovery within the first year following the wildfires whereas assemblage composition displayed significant changes that were apparent even in postfire year 5 (also see Richards and Minshall 1992). The changes wrought by fire affect macroinvertebrates in many more ways than just through alterations in food resources, e.g., via higher water temperatures. Individual life histories and lifestyles respond in different ways and to different degrees to these various changes. Opportunistic species, particularly those well suited for dispersal through drift and with relatively short generation times (such as chironomids and *Baetis*), seem to be especially adapted to conditions following fire, regardless of their trophic niche (Anderson 1992; Mihuc et al. 1996). In contrast, other species decreased in abundance soon after the fire and showed little or no recovery during this study. This was particularly noticeable among the Ephemeroptera, especially the dorsoventrally compressed taxa (e.g., *Cinygmula*, *Epeorus*, and *Rhithrogena*).

Our results emphasize the importance of studying stream ecosystems for a number of years following large-scale disturbance. Conclusions based on only one or a few years of data

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can be misleading in terms of overall trends, as evidenced, for example, by the apparent devastation of stream ecosystems immediately after the 1988 fires, their rapid progress toward recovery in postfire years 1 and 2, and their equally abrupt downturn in postfire years 3 and 4. Far too little data exist on conditions over extended periods after fire to know for certain whether the expectations for Yellowstone (Minshall et al. 1989; Minshall and Brock 1991) will prove correct. In fact, the initial recovery trajectory seen for Yellowstone streams is much different (faster initially, longer time delay before major storm impacts were seen, etc.) than expected based on the only other comparable study in central Idaho (Richards and Minshall 1992). The absence of comparable data on long-term effects, high year-to-year variability in postfire disturbance impacts among streams of different size, and differences in recovery trajectories compared with those found in other Rocky Mountain streams provide strong arguments for obtaining an extended temporal perspective for Yellowstone lotic ecosystems in the aftermath of the 1988 fires.

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Effects of climatic temperature change on growth, survival, and reproduction of rainbow trout: predictions from a simulation model[1]

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Abstract: The effects of changes in the annual temperature cycle on energy acquisition and the allocation of that energy to respiration, growth, and reproduction of female rainbow trout (*Oncorhynchus mykiss*) are evaluated using a new type of simulation model. We tested the effects of warmer (+2 and +4°C) and cooler (–2°C) water temperature cycles. We used Monte Carlo filtering techniques to define the range of parameter sets capable of generating realistic behaviour for individual female rainbow trout over a single reproductive cycle under the baseline temperature regime. The base temperature scenario generally resulted in maximum growth and reproductive success. Shifts in the annual temperature cycle of +2 and +4°C decreased growth and reproductive success, but most females were in better physiological condition. The –2°C scenario resulted in reduced growth, physiological condition, gonad index, and reproductive success. The effects of shifts in the annual temperature cycle varied substantially between individual model trout having relatively small differences in parameter values. These differences may be considered analogs of genotypic differences among individual trout in the field. The long-term implications of variability in physiological parameters between individuals are not easily evaluated experimentally. Models are an ideal tool for heuristically exploring such implications.

Résumé : Nous évaluons les effets des changements du cycle annuel de température sur l'acquisition d'énergie et l'allocation de cette énergie à la respiration, à la croissance et à la reproduction chez des truites arc-en-ciel (*Oncorhynchus mykiss*) femelles, à l'aide d'un nouveau type de modèle de simulation. Nous avons testé les effets de cycles thermiques de l'eau plus chauds (+2 et +4°C) et plus froids (–2°C). Nous avons eu recours aux méthodes de filtrage Monte Carlo pour définir la plage de séries de paramètres permettant de générer un comportement réaliste des individus femelles pendant un cycle de reproduction sous un régime thermique de base. C'est le scénario thermique de base qui donnait généralement le maximum de croissance et de succès de reproduction. L'accroissement du cycle annuel de température de +2 et +4°C faisait baisser la croissance et le succès de reproduction, mais la plupart des femelles se trouvaient en meilleure condition physiologique. Le scénario à –2°C occasionnait un ralentissement de la croissance, une baisse de la condition physiologique, de l'indice gonadique et du succès de reproduction. Les effets des modifications du cycle annuel de température variaient de façon importante dans le modèle entre des individus présentant des différences relativement faibles dans les paramètres. Ces différences peuvent être considérées comme analogues aux différences génotypiques entre les individus dans la nature. Les incidences à long terme de la variabilité des paramètres physiologiques entre individus sont difficiles à mesurer expérimentalement. Les modèles constituent un outil idéal pour explorer de façon heuristique ces incidences.
[Traduit par la Rédaction]

Introduction

Predicted gradual warming of the earth's climate would likely have effects at all levels of biological organization. Effects observed at higher levels of organization would reflect the net or integrated effects at lower levels. Fish population responses to warmer temperatures would be an emergent property of the varied direct responses of individual fish. DeAngelis and Cushman (1990) and Shuter and Meisner (1992) emphasize that linking simulation models, including physiological process models, across different levels of biological organization provides a powerful framework for assessing the effects of climate change.

Physiological process models have been used to predict the bioenergetic consequences for fish exposed to climatic temperature change associated with global warming. Temperature effects simulated include colder and longer winters, extended growing seasons, and hotter and longer summers (e.g., Hill and Magnuson 1990; Shuter and Post 1990). These analyses focused on how fish growth, survival, timing of spawning, prey

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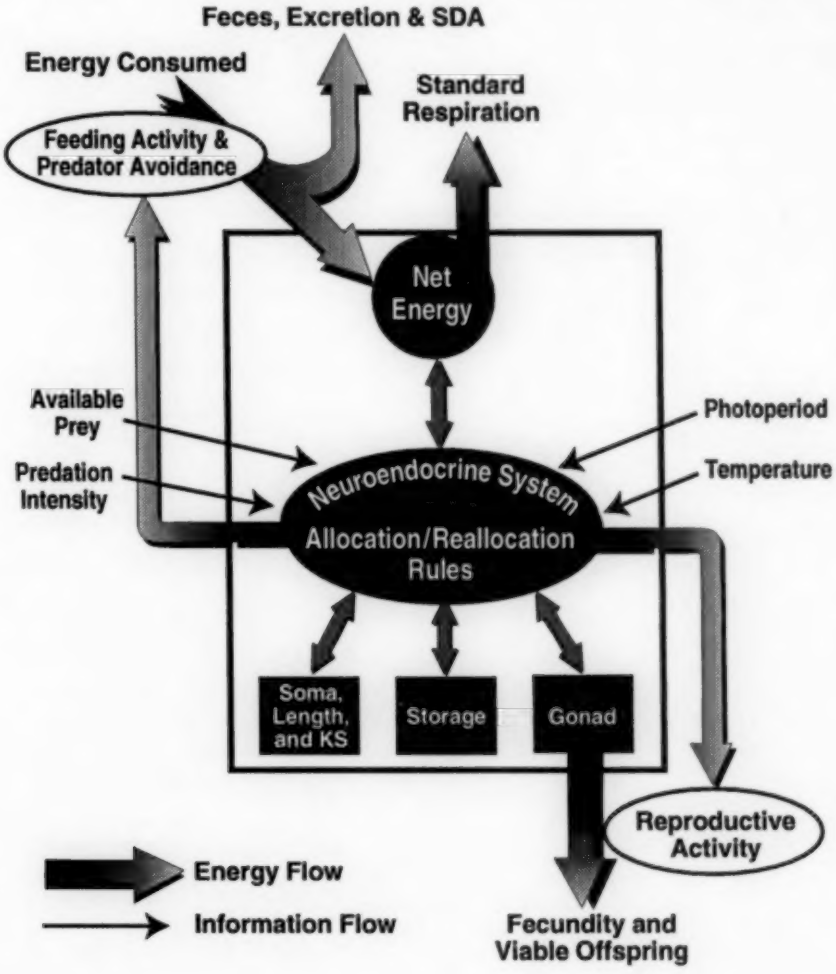
Fig. 1. Conceptual framework for the feedback system regulating the energy consumption of an adult rainbow trout and the allocation of that energy (*i*) to standard respiration, feeding activity predator avoidance, and reproductive activity and (*ii*) to and from soma, storage, and gonad compartments. Energy allocated to feeding activity, predator avoidance, and reproductive activity directly influences energy consumption, survival, and offspring viability, respectively. Energy allocated to soma is partitioned between increasing the index (KS) of physiological condition of the soma compartment and increasing length. Energy allocated to the gonad compartment determines fecundity (from Van Winkle et al. 1997).



consumption rates, habitat, and behavioral thermoregulation would respond to changes in temperature. These models have not, however, dealt with the potential effects of warmer temperature on the seasonally varying allocation of energy to respiration, growth, and reproduction over an annual cycle.

We use a simulation model to evaluate the effects of changes in the annual temperature cycle on energy acquisition and the allocation of that energy to respiration, growth, and reproduction of female rainbow trout (*Oncorhynchus mykiss*). The model is roughly configured for age-1 female rainbow trout in the Tule River, California (Studley et al. 1995). We use the model to test the effects of warmer water temperatures (+2 and +4°C), which crudely mimic changes predicted for global climate warming, and a cooler temperature (–2°C) scenario. A cooler temperature is included to establish model responses over a broader range of temperatures. Monte Carlo methods are used to randomly vary parameter values. We selected those parameter sets that allowed the female trout to satisfy our four calibration criteria involving length, condition, and reproductive success under baseline temperature conditions. We assumed that a comprehensive sample of parameter sets generated in this way would roughly bound the range of individual differences likely to arise from genetic variation in the baseline population. We used such samples to predict the range of individual responses to altered temperature regimes.

Description of model

The conceptual framework for our bioenergetics model was drawn from the literature on costs of reproduction, trade-offs, and fitness and literature on seasonal energy budgets, patterns

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Table 1. Initial conditions and parameter values for the reference female rainbow trout.

(A) Initial conditions on May 1 at the start of an annual reproductive cycle.

Variable	Value
Length (mm fork length), L	115
Index of physiological condition, KS	0.9
Energy content of the soma compartment (kcal), S	27.2[a]
Energy content of the storage compartment (kcal), A	0
Energy content of the gonad compartment (kcal), G	0

(B) Model parameters whose values were varied in Monte Carlo simulations.[b]

Name	Value	Definition and reference
Index of physiological condition		
KS_{spawn}	0.7	Minimum index of condition for spawning
KS_2	0.8	Minimum index of condition for starting into an annual reproductive cycle
KS_1	1.0	Index of condition below which consumption and respiration associated with foraging decrease, daily probability of mortality increases, and reallocation of soma to gonad when ΔE is negative decreases
Consumption		
a_C, b_C	0.58, 0.77	Parameters of weight-dependent function for consumption (Van Winkle et al. 1997; Rand et al. 1993); the parameter a_C has units of kcal of prey consumed per day, assuming 0.640 kcal·g^{-1} wet weight of natural invertebrate prey consumed (Hewett and Johnson 1992)
p	0.408	Scaling factor for consumption to reflect relative prey availability
T_{crit}	3.0°C	Lower critical temperature below which consumption is zero and respiration associated with foraging and reproductive activities is zero
(T_i, k_i)	3.5°C, 0.20 15.0°C, 0.80 22.5°C, 0.98 24.3°C, 0.20	Coordinates for the four points required by the Thornton and Lessem (1978) function for the temperature multiplier of maximum consumption (From and Rasmussen 1984; Van Winkle et al. 1997)
Respiration		
a_R, b_R	0.0071, 0.75	Parameters of the weight-dependent function for standard respiration (Stewart 1980; Van Winkle et al. 1997); the parameter a_R has units of kcal·day^{-1}
c_R	0.0693	Parameter of the temperature multiplier for standard respiration; corresponds to a Q_{10} of 2.0
r_1, r_2	1.0, 1.0	Multiple of standard respiration for foraging activity and reproductive activity, respectively
T_{crit}	3.0°C	Lower critical temperature below which consumption is zero and respiration associated with foraging and reproductive activities is zero
Mortality		
$F_{L_{max}}$	0.1	Value of the length-dependent term in the function for daily probability of mortality when the length of the trout is L_{max}
L_{min}, L_{max}	20, 200	Minimum and maximum length (mm) in the function for daily probability of mortality
Z_{act}	0.002 95	Daily instantaneous mortality rate for risks present primarily during the daily period of activity (number·day^{-1})
Z_{24}	0.002 95	Daily instantaneous mortality rate for risks present 24 h per day (number·day^{-1})
Energy allocation		
a_S, b_S	2.4×10^{-5}, 2.96	Parameters for the energy content of the soma compartment as a function of length; the value for the parameter a_L is based on a regression of wet weight of a rainbow trout versus its length and our assumption that 1.6 kcal·g^{-1} wet weight is a reasonable estimate of energy density for the soma compartment of rainbow trout age 1 and older

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Table 1 (*concluded*).

(B) Model parameters whose values were varied in Monte Carlo simulations.[b]

Name	Value	Definition and reference
a_A, b_A	0.09, 1.10	Parameters for the upper bound of the storage compartment as a function of the expected energy content of the soma compartment for a fish of length L (Brett et al. 1969; Elliott 1976)
a_G, b_G	0.09, 1.10	Parameters for the upper bound of the gonad compartment as a function of the expected energy content of the soma compartment for a fish of length L (Kamler 1992)
c_G	7.5	Rate constant in the function for reallocation of energy from soma to gonad
f_S, f_A, f_G		Monthly values for fraction of surplus energy allocated to soma, storage, and gonad, respectively

	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
Soma	1.0	0.8	0.8	0.8	0.8	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Storage	0.0	0.2	0.2	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0
Gonad	0.0	0.0	0.0	0.1	0.1	0.2	0.2	0.3	0.3	0.3	0.3	0.3

(C) Model parameters whose values were held constant in Monte Carlo simulations.

Name	Value	Definition and reference
Index of physiological condition		
KS_{min}	0.5	Minimum index of condition for survival (Shuter et al. 1980)
KS_{max}	1.3	Maximum index of condition for a rainbow trout inhabiting small streams
Consumption		
u_M	0.5	Predation intensity relative to a maximum value of 1.0, used in the equation for consumption
Energy allocation		
q_1	0.10	Fractional loss in reallocating energy from soma to respiration or from storage to gonad
q_2	0.05	Fractional loss in reallocating energy from storage to respiration
q_3	0.075	Fractional loss in reallocating energy from gonad to respiration
q_4	0.15	Fractional loss in reallocating energy from soma to gonad
Reproduction		
E_{egg}	79	Average energy content of a rainbow trout egg (cal) (Kamler 1992)
Other		
c_1, c_2, c_3	10.5, 6.5, 5.5	Coefficients of the function for the annual temperature cycle (Fig. 4c)
f_D	1.0 (May–Feb.) 0.5 (Mar.–Apr.)	Fraction of the daily period of activity the trout forages

Note: The absence of a literature reference for a parameter means that the value for that parameter is our best estimate based on evaluation of the literature, the values for related parameters, and the results of calibrating the model to our four acceptance criteria.

[a]Calculated as $S = KSa_S L^{b_S} = 0.9(0.000\,024)115^{2.96}$.

[b]The random values for some of the parameters listed in Table 1B were determined by the random values selected for some related parameter. For example, the randomly selected value for Z_{24} was assigned to Z_{act}, and values for (a_G, b_B) were assigned to (a_A, b_A). The 12 triplets of (f_S, f_A, f_G) values were determined for each female by fixing f_S at 1.0 for May; randomly selecting f_S values for June, August, October, and December; and then dividing the difference $(1.0 - f_S)$ equally between f_A and f_G or assigning all of the difference to f_A or f_G if the reference value for either of these two proportions was 0.0. We set the values for June = July, August = September, October = November, and December = (January, ..., April).

of energy allocation, and reproductive response variables. Our objective was to design a simulation model suitable for evaluating trade-offs among growth, survival, and reproduction in fish (Fig. 1). A general description of the model and a review of the supporting literature is given in Van Winkle et al. (1997); the following description is for a female rainbow trout. Parameter values and definitions are listed in Table 1.

Each model simulation tracks the daily consumption, respiration, growth, survival, and reproductive status of an individual female rainbow trout from May 1 to April 30. Length and the caloric mass of the soma, storage, and gonad compartments are followed separately as finite difference equations. For each day, length and soma are first used to compute an index of physiological condition. Next, soma, physiological condition, temperature, and prey availability are used to compute consumption and respiration rates. Surplus energy is computed as the difference between consumption, adjusted for other losses, and respiration. Month-specific fractions determine allocation of surplus energy to soma, storage, and gonad. Increased soma is then converted to length based on the physiological condition of the individual. Under conditions of negative surplus energy, the deficit is satisfied by reallocating energy from storage, soma, and (or) gonad. Thus, we allow for the option that energy demands placed on the storage compartment can be supplemented by the energy in both soma and gonad compartments. However, a model trout's exercising this option will have negative consequences for both individual survival and reproductive success. Probability of survival depends on both condition and length. Reproductive status also depends on physiological condition. Spawning occurs on April 30, provided condition has been maintained above a specified minimum. Gonad is converted to eggs upon spawning.

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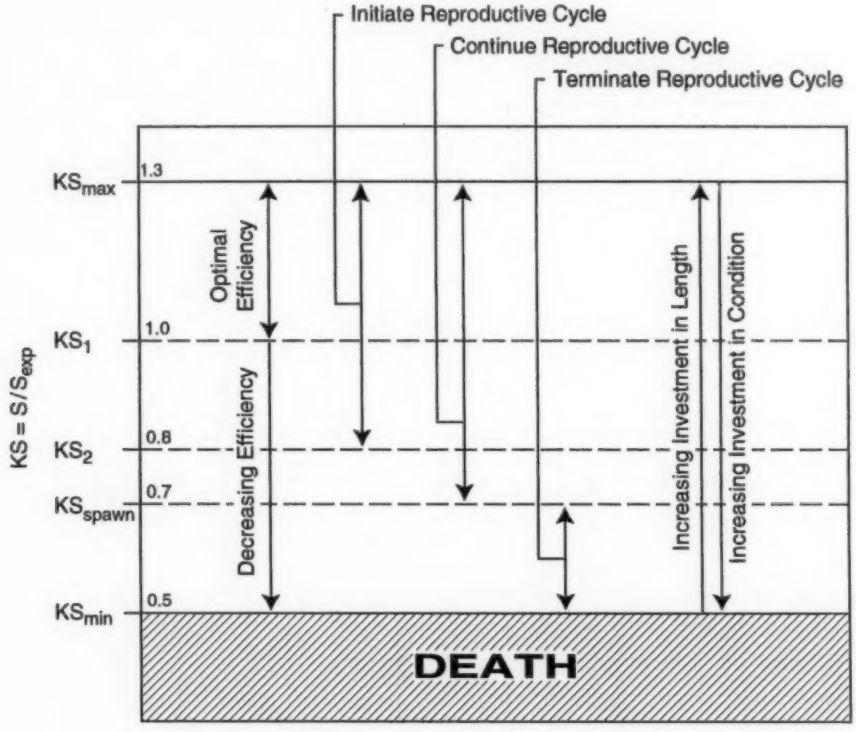
Fig. 2. Relationship between parameters defining the scale for the index of physiological condition (KS) and processes represented in the model. For a viable adult fish, KS can range between KS_{min} and KS_{max}. When $KS \geq KS_1$, the fish interacts with its environment with optimal efficiency. As KS decreases below KS_1, the fish interacts with its environment with decreasing efficiency. The reproductive cycle will be initiated only if $KS > KS_2$. As long as KS remains above KS_{spawn}, reproductive activities continue. If KS falls below KS_{spawn}, reproductive activities terminate. As KS increases toward KS_{max}, increases in soma are increasingly invested in increasing length, and as KS decreases toward KS_{min}, increases in soma are increasingly invested in improving or maintaining condition by increasing weight alone rather than increasing length.



Using updated values of length, soma, gonad, and storage, the computations are repeated for each day of a 1-year simulation.

Index of physiological condition

In our model, the index of physiological condition links the internal status of the animal to its reproductive choices and its ability to interact and survive in its environment (Fig. 2). For simplicity, we have defined this index solely in terms of the soma compartment. This definition raises the issue of how to link the storage and soma compartments of the model with measureable quantities in real fish. Some body components can be easily associated with specific model compartments. For example, short-term energy storage materials such as liver glycogen would belong in the storage compartment, while skeletal materials, muscle proteins, and structural lipids would belong in the soma compartment. Nonstructural lipids could be assigned to either compartment, depending on the influence they have on the behavioral changes summarized in Fig. 2. This assignment could vary considerably from species to species and, in some cases, might require a more complex representation in the model of the feedback links between behaviour and body composition.

We define the index of physiological condition (KS) as the energy content of the soma compartment (S; kcal (1 kcal = 4.18 kJ)) divided by that expected (S_{exp}) for a healthy trout of the same length (L; mm):

$$(1) \quad KS = \frac{S}{S_{exp}}$$

where S_{exp} is the soma predicted from length ($= a_S L^{b_S}$). We specify five benchmarks along the KS scale (Fig. 2). KS_{min} represents the minimum physiological condition for survival; a model rainbow trout dies if $KS < KS_{min}$. We view KS_{min} as an autecological threshold independent of the biotic environment. KS_{max} represents the maximum physiological condition for a rainbow trout inhabiting a specific type of ecosystem. We view KS_{max} as a synecological upper threshold that reflects the environmental setting or type of ecosystem. For example, KS_{max} may differ for a rainbow trout inhabiting a stream, a lake, or a hatchery. KS_1 is an intermediate condition threshold between KS_{min} and KS_{max}, below which we assume that both consumption and respiration associated with foraging decrease and that risk of mortality increases; the relationship between length and soma that is consistent with $KS = KS_1$ is given by $S = a_S L^{b_S}$. KS_2 is the minimum physiological condition required to initiate the annual reproductive cycle. Finally,

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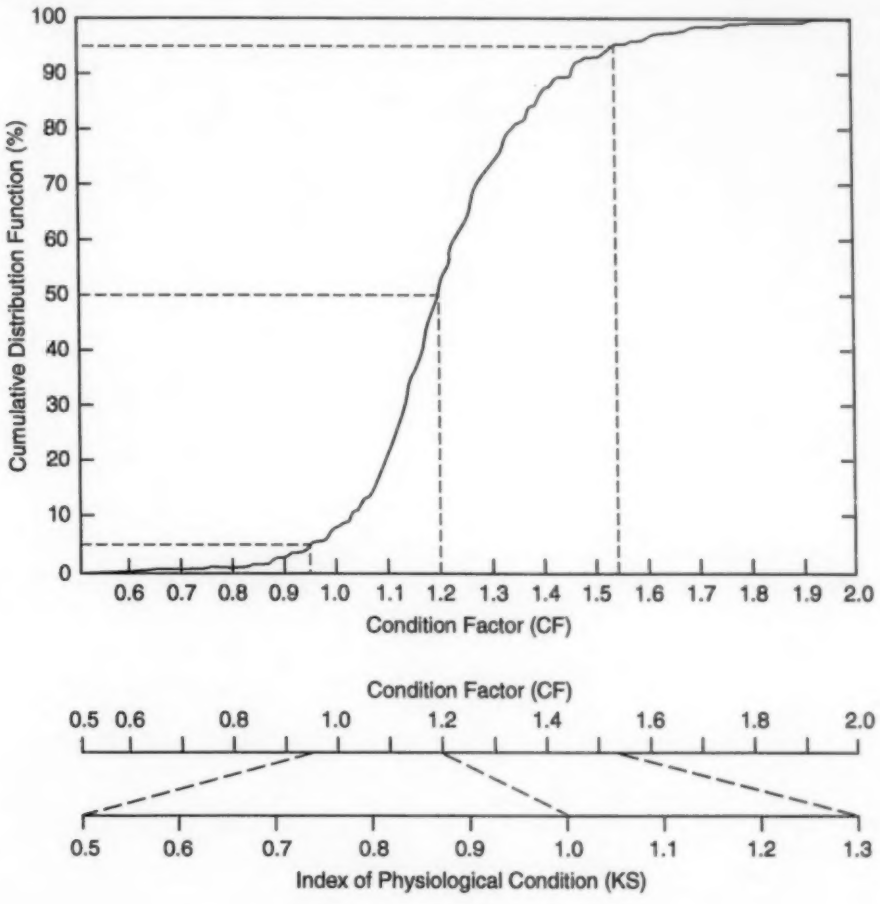
Fig. 3. Cumulative frequency distribution (CDF) of condition factor (CF) values for all ages of rainbow trout collected from the Tule River, California, in September and October of 1987–1993. A hypothetical but reasonable correspondence between this CF scale and the KS scale used in the model is indicated.



KS_{spawn} is the minimum physiological condition below which energy is no longer allocated to gonad, energy already in the gonad compartment is reallocated to respiration, and the annual reproductive cycle is aborted.

To be consistent conceptually with our bioenergetics framework (Fig. 1), our formulations given below for consumption, respiration, and length depend on the energy content of the soma compartment and its index of physiological condition (KS), rather than on the wet weight of the entire fish and the traditional condition factor (CF). However, KS values can be related to CF values. CF is usually defined as the wet body weight of a fish divided by that expected for the average fish of the same length, where the expected weight is estimated either as L^3 or from a log–log regression of wet weight on length. We used length, weight, and CF data for rainbow trout from the Tule River, California (Studley et al. 1995), to derive a relationship between CF and KS. CF values were calculated as $(W \times 10^5)/L^3$, where W is wet weight in grams and L is fork length in millimetres. We pooled CF data from the fall samples for all ages for the years 1987–1993 (Fig. 3; $N = 2529$). As a hypothetical but reasonable correspondence, we equate the median CF value (= 1.20) with $KS_1 = 1.0$, the 5th percentile CF value (= 0.95) with $KS_{min} = 0.5$, and the 95th percentile CF value (= 1.54) with $KS_{max} = 1.3$ (Fig. 3).

Consumption

Maximum consumption rate (C_{max}; kcal·day^{-1}) is represented as a function of soma and temperature (T):

$$(2) \quad C_{max} = \begin{cases} a_C S^{b_C} f(T), & T \geq T_{crit} \\ 0, & T < T_{crit} \end{cases}$$

where (a_C, b_C) determine the dependence of maximum consumption on soma, and T_{crit} is the lower critical temperature below which feeding ceases. The temperature multiplier, $f(T)$, has a value of 1.0 in the temperature range optimal for feeding and decreases at higher and lower temperatures (Hewett and Johnson 1992). Realized consumption on any given day (C; kcal·day^{-1}) is calculated as a fraction of maximum consumption:

$$(3) \quad C = I_{KS}(1.0 - u_M)pf_D DC_{max}$$

where I_{KS} is the effect of physiological condition, u_M is a

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constant predation intensity, p is a constant prey availability, D is hours of the day trout are active, and f_D is the proportion of D spent foraging. For rainbow trout, D is a function for hours of daylight, which varies as a function of calendar day and latitude (Brock 1981), plus the hours before sunrise and after sunset. Realized consumption increases with condition and prey availability (p) and decreases with predation intensity (u_M). I_{KS} is assumed 1.0 for $KS > KS_1$, and linearly declines from 1.0 to 0.0 as KS declines from KS_1 to KS_{min}. Fish in poorer condition typically reduce either the time spent foraging or their swimming speed during foraging (Goolish 1992). Consumption decreases with increasing u_M to reflect the disruption of foraging activities caused by increased predator activity.

Respiration

Total respiration is represented as the sum of three terms: standard or resting respiration, activity respiration associated with foraging and predator avoidance, and activity respiration associated with reproduction. Standard respiration (R_{std}; kcal·day^{-1}) increases with soma and temperature:

$$(4) \quad R_{std} = a_R S^{b_R} e^{c_R T}$$

where (a_R, b_R) and c_R determine the dependence of standard respiration on soma and temperature, respectively (Hewett and Johnson 1992). Total respiration (R; kcal·day^{-1}) is computed from R_{std} as

$$(5) \quad R = \begin{cases} R_{std} + I_{KS} r_1 f_D D R_{std} + r_2 (1.0 - f_D) D R_{std}, & T \geq T_{crit} \\ R_{std}, & T < T_{crit} \end{cases}$$

where r_1 and r_2 are the multipliers of R_{std} for foraging and predator avoidance and for reproduction, respectively, and $(1.0 - f_D)$ is the proportion of the active period the trout spends in reproductive activities such as migration, courtship, redd construction, and spawning. As with consumption, we assume the rainbow trout is inactive at temperatures less than T_{crit}. Respiration associated with foraging and reproduction apply to the portion of the active period spent in each of these activities. Values for r_1 and r_2 are defined for a healthy rainbow trout, i.e., $KS \geq KS_1$ (Fig. 2). The I_{KS} function reduces activity respiration associated with foraging and predator avoidance for trout in poor condition. The I_{KS} function, however, does not appear in the third term because activity respiration associated with reproduction is assumed to be independent of physiological condition for an individual that spawns.

Energy allocation and transfer

We assume that 40% of the energy consumed each day is lost to egestion, excretion, and specific dynamic action (Van Winkle et al. 1997). Thus, surplus energy (ΔE; kcal·day^{-1}) is

$$(6) \quad \Delta E = 0.6C - R$$

Allocation of ΔE to and from soma, storage, and gonad is conditioned by two demand functions: respiration and gonad development. Respiratory demand (eq. 5) is satisfied if $\Delta E > 0$. If respiratory demand exceeds $0.6C$, energy is transferred first from storage, then from soma, and as a last resort from gonad. Respiratory demand must be satisfied, even if it results in eventual death (i.e., $KS \leq KS_{min}$).

Energy demand for gonad development is satisfied after respiratory demand is met and involves specifying a target gonad at spawning based on length of the female at the end of the growing season. We assume that no increase in length occurs from the end of the growing season (t_1; the first day that $\Delta E < 0$) until the day of spawning (t_{sp}; April 30). On day t_1, we calculate the expected value of soma on the day of spawning ($S_{exp}(t_{sp})$; kcal) based on length on day t_1:

$$(7) \quad S_{exp}(t_{sp}) = a_S L(t_1)^{b_S}$$

where a_S and b_S are parameters that relate soma to length under the condition that $KS = KS_1$. We next calculate the expected value for gonad on the day of spawning ($G_{exp}(t_{sp})$; kcal) from soma:

$$(8) \quad G_{exp}(t_{sp}) = a_G S_{exp}(t_{sp})^{b_G}$$

where a_G and b_G are parameters relating gonad to soma. We treat $G_{exp}(t_{sp})$ as a target value for the gonad compartment on the day of spawning. The daily rate of energy allocation to the gonad compartment during the period t_1 to t_{sp} required to attain G_{exp} on day t_{sp} is computed. The actual rate of allocation to gonad depends on the physiological condition of the female and may be less. The source of the energy to be allocated to gonad on a given day depends on that day's values for surplus energy (ΔE), storage, and KS. Gonad index on any day t is defined as $G(t)/G_{exp}(t_{sp})$.

When ΔE is positive, month-specific fractions determine how this energy is allocated to each compartment:

$$(9) \quad \begin{aligned} S(t+1) &= S(t) + f_S(t)\Delta E(t) \\ A(t+1) &= A(t) + f_A(t)\Delta E(t) \\ G(t+1) &= G(t) + f_G(t)\Delta E(t) \end{aligned}$$

where $f_S(t)$, $f_A(t)$, and $f_G(t)$ are the fractions of surplus energy allocated to soma, storage, and gonad compartments, respectively. We set upper bounds each day on the energy content of the storage compartment (A_{max}) and gonad compartment (G_{max}) based on S_{exp} (eq. 7):

$$(10) \quad \begin{aligned} A_{max} &= a_A S_{exp}^{b_A} \\ G_{max} &= a_G S_{exp}^{b_G} \end{aligned}$$

Energy allocated to storage or gonad in excess of the upper bound is allocated to soma instead.

When ΔE is negative, the energy demand of the gonad compartment is satisfied by transferring energy from storage first and soma second, and then the energy content of each compartment is updated. There is a fractional energy loss for transferring energy from any of the three compartments to respiration due to the catabolic cost of breaking down existing biomass and a greater fractional energy loss for transferring energy from storage or soma to gonad (Van Winkle et al. 1997).

Mortality

Risks of mortality are divided into two categories: those present 24 h a day (e.g., disease and parasites) and those present primarily when rainbow trout are active (e.g., predation). We assume that mortality risk decreases with condition (Fig. 2) and size. Also, a rainbow trout dies when its condition falls below a minimum threshold (KS_{min}). Daily probability of mortality (P_{mort}) is

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$$(11)\quad P_{\text{mort}} = \begin{cases} 1.0 - e^{-Z}, & \text{KS}_{\min} < \text{KS} \le \text{KS}_{\max} \\ 1.0, & \text{KS} \le \text{KS}_{\min} \end{cases}$$

where Z is the total daily mortality rate (number·day^{-1}). Z is the sum of mortality rates for those risks always present (Z_{24}) and for those risks present during activity (Z_{act}):

$$(12)\quad Z = F_{\text{KS}} F_{\text{L}} (Z_{24} + DZ_{\text{act}})$$

The factors F_{KS} and F_{L} are the condition-dependent and length-dependent adjustments to mortality rate, respectively, and decrease with increasing condition and length as follows:

$$(13)\quad F_{\text{KS}} = \begin{cases} \dfrac{\text{KS}_1 - \text{KS}_{\min}}{\text{KS} - \text{KS}_{\min}}, & \text{KS}_{\min} < \text{KS} < \text{KS}_1 \\ 1.0, & \text{KS}_1 \le \text{KS} \le \text{KS}_{\max} \end{cases}$$

$$(14)\quad F_L = \begin{cases} 1.0, & L \le L_{\min} \\ 1.0 - \left(\dfrac{1.0 - F_{L_{\max}}}{L_{\max} - L_{\min}}\right)(L - L_{\min}), & L_{\min} < L \le L_{\max} \\ F_{L_{\max}}, & L > L_{\max} \end{cases}$$

where $F_{L_{\max}}$ is the minimum value for the length-dependent factor that applies to trout larger than $L_{\max}$.

Trade-off between condition and length

There are trade-offs for a trout between using surplus energy to improve physiological condition or to increase body length. More investment in condition rather than length results in increases in both consumption (eq. 3) and respiration (eq. 5). Increasing physiological condition and increasing length both decrease the daily probability of mortality (eqs. 13 and 14) but not necessarily by the same amount.

We assume that, as condition increases, trout use surplus energy more for increasing their length. At time $t + 1$, we calculate $S(t + 1)$ (eq. 9), and from $S(t + 1)$ we update the value for KS and then the value for L. When soma increases, we assume that the index of condition does not decrease. The maximum value for an updated KS value (MAXKS) would occur if length does not increase (i.e., $L(t + 1) = L(t)$):

$$(15)\quad \text{MAXKS}(t+1) = \begin{cases} \dfrac{S(t+1)}{S_{\text{exp}}[L(t)]}, & \text{MAXKS}(t+1) \le \text{KS}_{\max} \\ \text{KS}_{\max}, & \text{MAXKS}(t+1) > \text{KS}_{\max} \end{cases}$$

where $\text{KS}_{\max}$ is the maximum value allowed for physiological condition (Fig. 2). A rainbow trout in poor condition would likely repair existing soma (e.g., muscle mass) rather than increase its length. The change in condition from one day to the next is represented as

$$(16)\quad \text{KS}(t+1) = \text{KS}(t) + \frac{\text{KS}_{\max} - \text{KS}(t)}{\text{KS}_{\max} - \text{KS}_{\min}} \times (\text{MAXKS}(t+1) - \text{KS}(t))$$

The quantity $\text{MAXKS}(t + 1) - \text{KS}(t)$ is the maximum possible daily increase in KS. When KS is close to $\text{KS}_{\min}$, most of this maximum increment in KS is realized. As the rainbow trout's condition improves ($\text{KS} - \text{KS}_{\max}$), more of any new surplus is used to increase length. Given an update condition, length is then calculated as

$$(17)\quad L(t+1) = \left(\frac{S(t+1)}{a_S \text{KS}(t+1)}\right)^{\frac{1}{b_S}}$$

When surplus energy is negative and once the storage compartment has been depleted, soma and KS decrease, but length remains the same.

Reproduction and index of reproductive success

Simulations presented in this paper begin with individuals that are sexually mature whose physiological condition exceeds the minimum required to initiate the annual reproductive cycle (KS_2). A model rainbow trout spawns on April 30 if its index of physiological condition remains above a minimum (KS_{spawn}) throughout the simulation (Fig. 2). If KS drops below KS_{spawn} on any day, the rainbow trout does not spawn. When a rainbow trout does not spawn, the energy in the gonad compartment is reallocated to respiration over a period of days. Fecundity (F) is calculated as

$$(18)\quad F = \frac{G(t_{\text{sp}})}{E_{\text{egg}}}$$

where $G(t_{\text{sp}})$ is the energy content (kcal) of the gonad on April 30, and E_{egg} is the energy content of a single egg (kcal per egg). The index of reproductive success (I_{repro}) is computed as the product of the probability the female survives to spawn (P_s) and the number of eggs spawned (F):

$$(19)\quad I_{\text{repro}} = P_s F$$

Methods

Reference calibration

Reference parameter values (Table 1) were selected that resulted in a model simulation satisfying the following criteria: (*i*) growth of 45 ± 2 mm (i.e., from 115 to 158–162 mm) from May 1 to April 30, (*ii*) physiological condition on April 30 of 0.90 ± 0.05, (*iii*) gonad index at the end of growing season (the first day that $\Delta E < 0$) of 0.70 ± 0.03, and (*iv*) index of reproductive success of 70 ± 5 eggs. The length criterion was based on data for rainbow trout in the Tule River, California (Studley et al. 1995). The other three criteria are based on a synthesis of information in the literature and experience with the model (Van Winkle et al. 1997).

Monte Carlo filtering

Monte Carlo filtering is a procedure for objectively estimating realistic variability around parameter values and improving confidence in model predictions (Rose et al. 1991). Filtering involves (*i*) use of available information to establish criteria for acceptable model behavior, (*ii*) application of Monte Carlo methods to randomly vary parameters over a range of values to generate a set of model predictions, and (*iii*) classification of each simulation as acceptable or unacceptable based on whether it satisfies the criteria. Acceptable model simulations can be regarded as equally valid sets of realistic parameter values. Operationally, the filtering procedure acts much like natural selection, and hence, we regard each acceptable simulation as representing an individual female rainbow trout with a unique genotype

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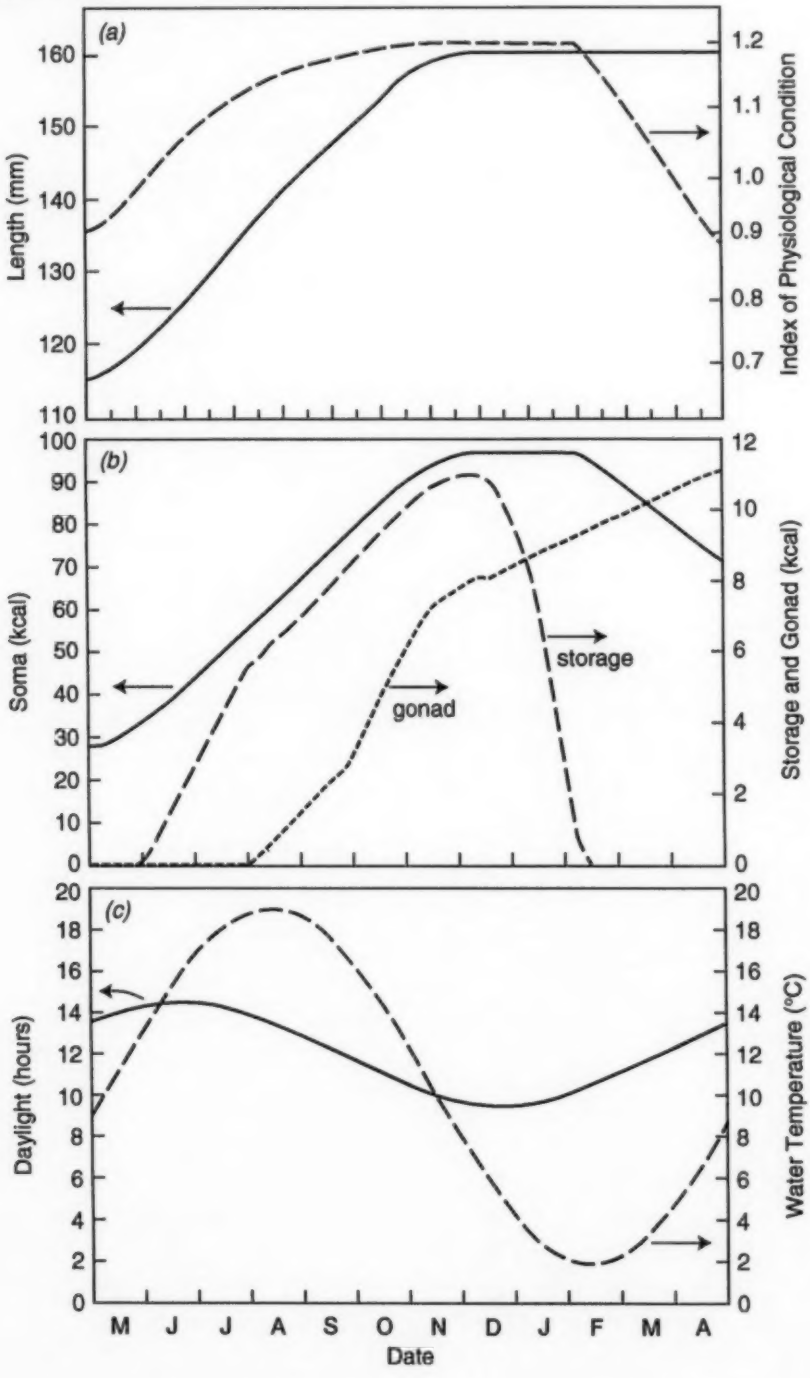
Fig. 4. Model results for one annual reproductive cycle for (*a*) length and index of physiological condition and (*b*) energy content of the soma, storage, and gonad compartments of a female rainbow trout, using the reference set of parameter values (Table 1). The annual daylight cycle (Brock 1981) and temperature cycle are shown in Fig. 4*c*.



characterized by its set of parameter values. A stratified sampling design, Latin hypercube sampling, was used to ensure thorough representation of parameter distributions (Rose et al. 1991).

Minimum and maximum values were specified for each of the four acceptance criteria. Wider ranges were used with the acceptance criteria for filtering than for calibration: 160 ± 15 mm for length;

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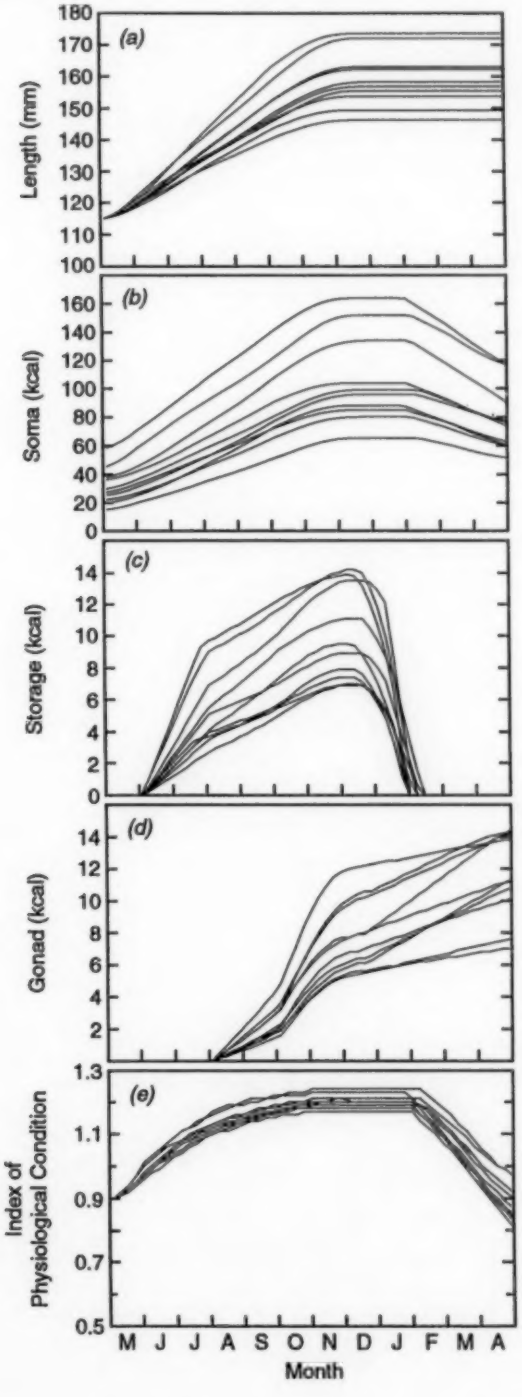
Fig. 5. Results from the first 10 of the 110 acceptable simulations illustrating typical variability in predicted values for (*a*) length, (*b*) soma, (*c*) storage, (*d*) gonad, and (*e*) index of physiological condition.



0.90 ± 0.10 for index of physiological condition; 0.70 ± 0.20 for gonad index; and 70 ± 25 for index of reproductive success. We simulated 5400 female rainbow trout using parameter values drawn from uniform distributions with lower and upper limits equal to ±10% of the reference parameter values (Table 1B). Previous analysis of the rainbow trout model showed that it was unlikely that most parameters varied more than ±10% of their reference values because >10% variation resulted in unrealistic simulations. Uniform distributions were used to ensure that all parameter values were equally likely; we had no basis for specifying modes of distributions within the ±10% range. Each of the 5400 simulations was classified as acceptable or unacceptable. We consider the set of acceptable simulations as a sample of female rainbow trout with realistic parameter values governing allocation and transfer of energy and mortality over the course of an annual cycle. Given the large number of simulations (5400) relative to the number of parameters varied (33), it seems unlikely that types of acceptable parameter combinations have been inadvertently rejected because of stochastic effects of mortality.

Model predictions and associated parameter values for acceptable simulations were analyzed. For a subset of the acceptable simulations, model predictions of daily values of length, soma, storage, gonad, and condition are presented. The importance of model parameters in determining whether a simulation was acceptable was assessed by comparing cumulative distribution functions (CDFs) for acceptable and unacceptable simulations. If the distribution functions for a parameter differ between acceptable and unacceptable simulations, then that parameter is important. We also computed all pairwise correlations among parameters for acceptable simulations to see if filtering induced any covariances among parameter values. Correlation coefficients (r) whose absolute values were >0.3 were deemed significant (equivalent to $p < 0.01$ for $N = 110$, the number of acceptable simulations).

Altered temperature scenarios

The simulated temperature regime for the baseline scenario is for the study reach in the Tule River (Studley et al. 1995). The minimum temperature (2.0°C) occurs on February 10, and the maximum temperature (19.0°C) occurs on August 11 (Fig. 4*c*). We compared model predictions under the baseline scenario with both a cooler water temperature scenario (–2°C every day) and warmer temperature scenarios (+2°C and +4°C every day). For the baseline and each altered temperature scenario, 110 simulations were performed corresponding to each of the acceptable sets of parameter values. CDFs of predicted values of the four criteria, and the total number of eggs spawned by survivors ($\sum I_{repro}$), are presented for the baseline and altered temperature scenarios. Only female trout that spawned were included in the CDFs. The role of individual-level variability was demonstrated by showing how small differences in parameter values can lead to very different survival and growth responses to altered temperatures.

Results

Reference calibration

The reference set of parameter values (Table 1) generated realistic model predictions (Fig. 4). Length increased from 115 to 160 mm, with no further growth after the end of growing season in December. Condition, soma, and storage peaked at the end of the growing season and declined after that as energy was transferred to respiration and gonad. Gonad increased beginning in September, reaching 0.72 by the end of the growing season. Gonad continued to grow from December through April because of transfer of energy, first from the storage compartment and then, starting in February, from the soma compartment. Index of reproductive success was 71 eggs (142 eggs × 0.5 probability of survival).

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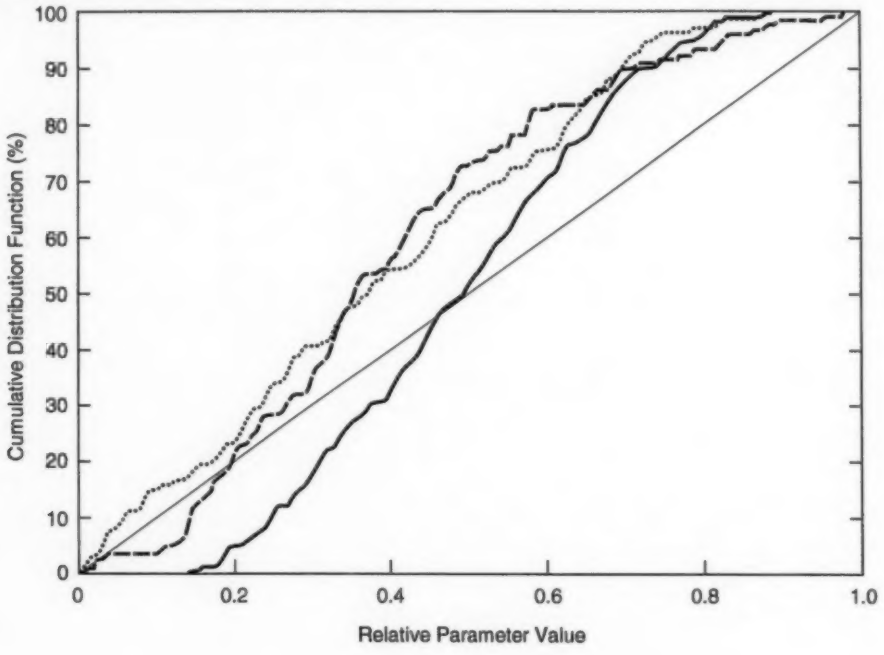
Fig. 6. Cumulative distribution functions for the 110 acceptable simulations for the three parameters whose values were most important in determining the acceptability of a simulation: exponent relating soma to length (heavy solid line), exponent relating maximum consumption to soma (broken line), and exponent relating standard respiration to soma (dotted line). To facilitate comparison, values for all three parameters have been rescaled to range between 0.0 to 1.0. The cumulative distribution functions for the 5290 unacceptable simulations for all three parameters are the same straight line between (0, 0%) and (1.0, 100%).



The parameters that proved most effective in calibrating the model to the length and condition criteria were those that influence the trade-off between energy acquisition (b_C in eq. 2 and p in eq. 3) and energetic costs (b_R and r_1 in eqs. 4 and 5). The values for the fraction of surplus energy allocated to soma, storage, and gonad during the months of June through November were most effective in calibrating to the gonad criterion. Once the criteria for length, condition, and gonad index were satisfied, Z_{24} (eq. 12) was adjusted to obtain an annual probability of survival of 0.50.

Monte Carlo filtering

One hundred and ten cases of the 5400 simulated females satisfied all four acceptance criteria. Of the remaining 5290 females having unacceptable sets of parameter values, 1060 died of starvation before spawning, and 894 did not spawn because they were in poor condition. While the gonad, soma, and storage varied widely among acceptable simulations, length and condition were similar (Fig. 5).

Three parameters were of primary importance in determining whether a simulation was acceptable: the exponent relating soma to length (eq. 7), the exponent relating maximum consumption to soma (eq. 2), and the exponent relating standard respiration to soma (eq. 4) (Fig. 6). For the other 30 parameters that were varied, the CDFs and medians for acceptable compared with unacceptable cases were not statistically different ($P > 0.05$; Kolmogorov–Smirnov test and median test for two samples).

Five significant correlations ($|r| > 0.3$) between parameters were detected for the acceptable simulations. The exponents relating soma to length (eq. 7) and gonad to soma (eq. 8) were negatively related ($r = -0.68$), demonstrating the balance between these two parameters in satisfying the gonad index criterion. The exponent relating maximum consumption to soma (eq. 2) was negatively correlated with the intercept parameter in that equation ($r = -0.39$) and with one of the parameters defining the temperature-dependent multiplier of maximum consumption ($r = -0.45$). In both cases, acceptable bounds on the pattern of consumption were achieved by either increasing the exponential parameter and decreasing the intercept and temperature parameter or vice versa. The exponent relating maximum consumption to soma (eq. 2) was positively correlated with the exponent relating standard respiration to soma (eq. 4; $r = 0.44$) and with the parameter in the temperature-dependent multiplier of standard respiration (eq. 4; $r = 0.30$). These two correlations illustrate that acceptable bounds on the balance between consumption and respiration were achieved by increasing (decreasing) both consumption and respiration.

Altered temperature scenarios

Temperature changes had a marked effect on survival, length, physiological condition, gonad index, and index of reproductive success (Fig. 7). All 110 females survived and spawned with the baseline, –2, and +2°C temperature scenarios; 30 females died and one female survived but did not spawn with the +4°C scenario.

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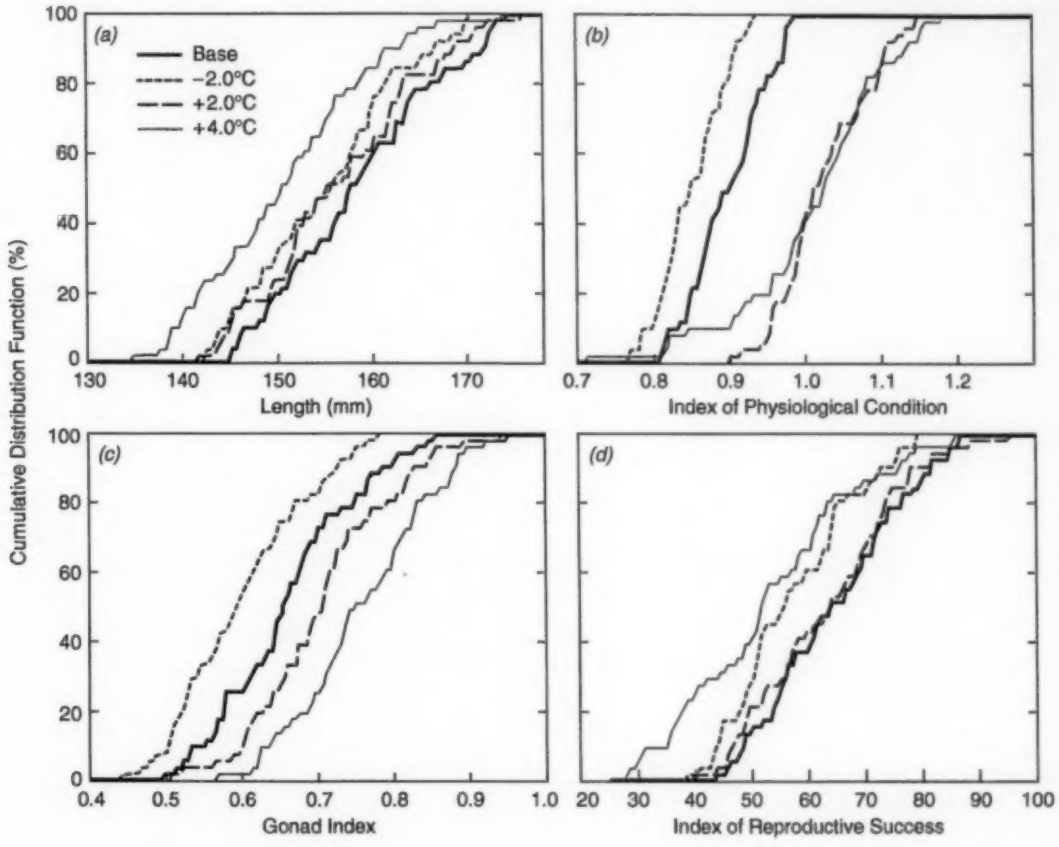
Fig. 7. Comparison of the cumulative distribution functions (CDFs) for the base temperature scenario and the three altered temperature scenarios for (*a*) length of the trout on day 365, (*b*) index of physiological condition on day 365, (*c*) gonad index, and (*d*) index of reproductive success.



Length

Spawners were shorter under all three altered temperature scenarios compared with baseline (Fig. 7*a*). CDFs were roughly parallel for the four temperature conditions. Median lengths were 159 mm (baseline), 155 mm (–2 and +2°C), and 150 mm (+4°C). Decreased growth resulted from a shift in the balance between consumption and respiration with temperature scenarios cooler and warmer than the baseline scenario, resulting in less surplus energy during the growing season (eq. 6). This shift was increased by the interaction between the temperature regime and the monthly parameter values specifying the proportion of energy allocated to soma, i.e., 1.0 in May, 0.8 in June through September, and 0.7 from October on. For example, with the –2°C scenario, temperatures in May were considerably less than the optimum for growth (15°C; Brett 1979) when all surplus energy was allocated to soma, and then when the temperature increased in June–September to 14–17°C, only 80.0% of the surplus energy was allocated to soma.

Index of physiological condition

Compared with baseline conditions, KS values were consistently lower with the –2°C scenario and consistently higher with the +2 and +4°C scenarios (Fig. 7*b*). This pattern resulted because the end of the growing season varied with the temperature scenario from an average of December 1 with the –2°C scenario to February 18 with the +4°C scenario. Energy is not reallocated from soma to satisfy the gonad demand until after the end of the growing season and after all energy in storage has been used to satisfy respiration and gonad demands. The average date when the storage compartment reached zero varied from January 18 with the –2°C scenario to March 14 with the +4°C scenario. Thus, as the temperature scenario becomes warmer, the energy in the soma compartment is spared until later in the winter, and the index of physiological condition on the day of spawning remains higher. The results with the +4°C scenario, however, indicate that there was a limit. Thirty of the 110 females died with this warmest scenario due to poor condition, and some of the remaining individuals were in marginal condition by the end of the simulation, as indicated by the long left tail of the KS CDF with the +4°C scenario (Fig. 7*b*). Some of the deaths due to starvation occurred during August and September, while others occurred as it warmed up the following spring.

Gonad index

Gonad index increased with increasing temperature (Fig. 7*c*).

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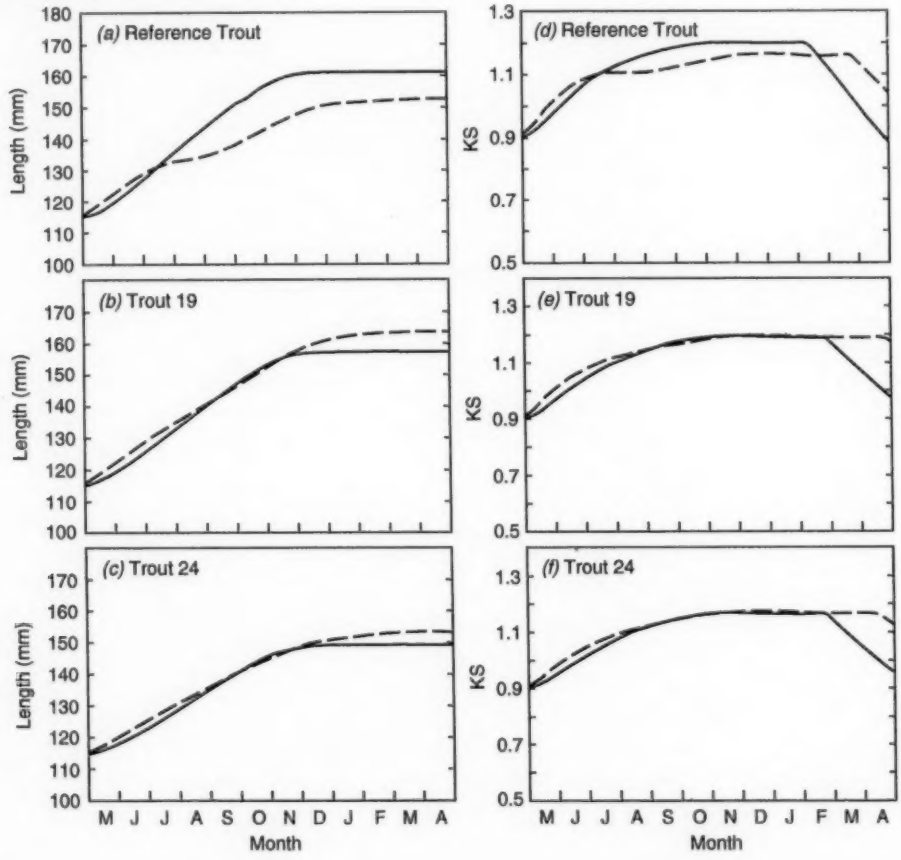
Fig. 8. Differences in how three trout responded to the +4°C temperature scenario (broken lines) as compared with the base temperature scenario (solid lines). Time series of predicted values for an annual reproductive cycle are given for length (*a–c*) and for the index of physiological condition (*d–f*) for the reference trout, which did less well at +4°C than at the base scenario, and for trout 19 and trout 24, which did better at +4°C than at the base scenario.



Median values were 0.61 (–2°C), 0.68 (base), 0.73 (+2°C), and 0.78 (+4°C). This pattern, as for KS, reflects that the end date for the growing season follows the same pattern, i.e., December 1 (–2°C), December 16 (base), January 8 (+2°C), and February 18 (+4°C). The longer growing season allows for greater consumption and for more of the energy consumed to be allocated to gonad.

Index of reproductive success and total egg production

Indices of reproductive success (IRS) were similiar with the baseline and +2°C scenarios and lower with the –2°C and the +4°C scenarios (Fig. 7*d*). Median values were 59 (–2°C), 70 (base and +2°C), and 54 (+4°C). The lower IRS values were due to decreases in both fecundity and the annual probability of survival (eq. 19). Total eggs produced by the females that survived and spawned were 12 980 (–2°C), 14 080 (baseline), 13 530 (+2°C), and 8611 (+4°C).

Variability among individuals

Trout responses to altered temperatures varied greatly among individuals. The pattern was more complex and interesting than a simple shift in frequency distributions such that each female retained the same relative position within the frequency distribution. For example, there were five females that where larger and in better condition on April 30 at the +4°C scenario than at the other three temperature scenarios. Comparison of growth curves at the baseline and +4°C scenarios for the reference trout and two of these five females indicate that trout Nos. 19 and 24 maintained their growth rate during the high temperatures in July–September (Figs. 8*a*–8*c*). KS values for the reference trout at +4°C dropped below those at the baseline temperatures starting in the summer and declined starting in late March (Fig. 8*d*). The KS values for trout Nos. 19 and 24 at +4°C were very similar to those at the baseline temperature scenario until March, but they did not begin declining until mid-April.

Slight, but important, differences in the temperature multipliers of maximum consumption and standard respiration account for why, in comparing the base and +4°C scenarios, some females died in the warmer scenario while others grew larger and were in better condition. For the 30 females that died in the +4°C scenario, the temperature multiplier for consumption peaked below 20.0°C and decreased rapidly at higher temperatures (Fig. 9). Thus, during the period of early

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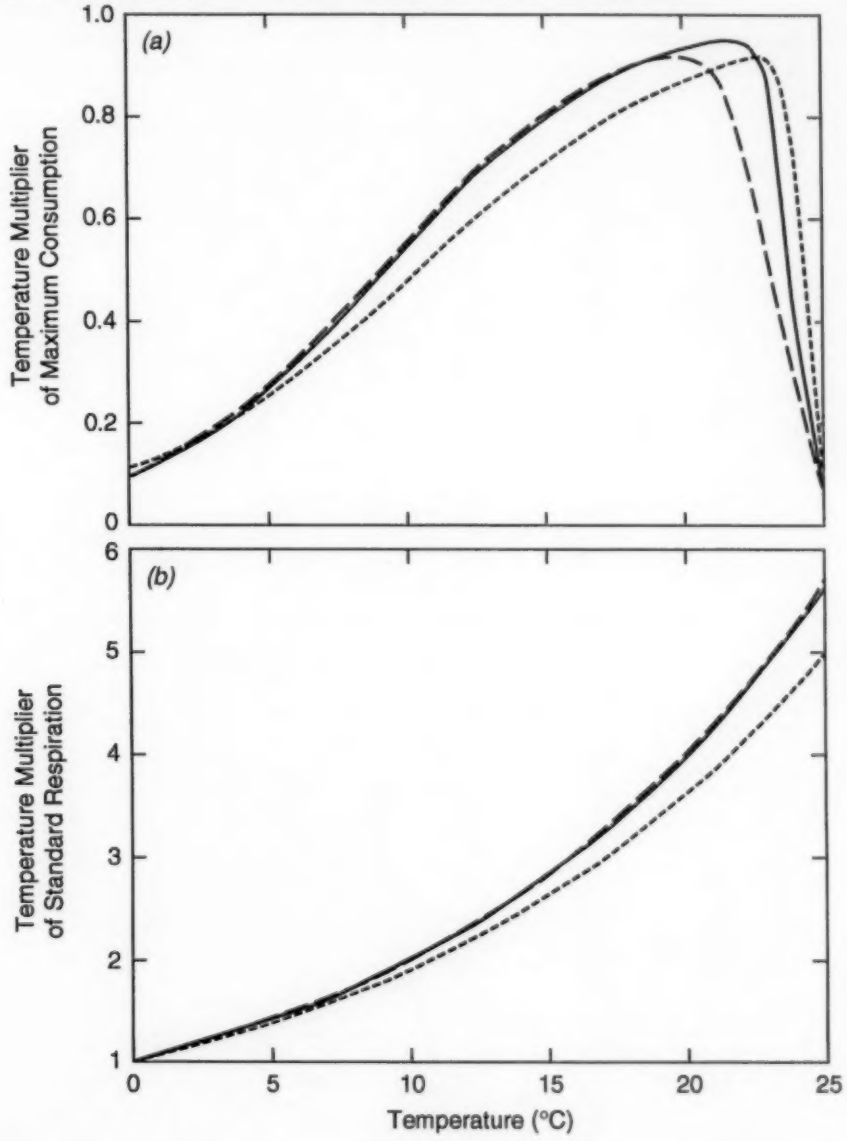
Fig. 9. Dependence of (*a*) maximum consumption and (*b*) standard respiration on temperature for the reference trout that did less well at the +4°C temperature scenario than at the base scenario (solid line), for the average of the 5 trout that did better at the +4°C scenario than at the base scenario (dotted line), and for the average of the 30 trout that died at the +4°C scenario (broken line).



July to mid-September when the temperature exceeded 20.0°C in the +4°C scenario, energy consumption for each of these females was greatly reduced leading to increased mortality. For the five females that grew larger and were in better condition on April 30, both temperature multipliers were slightly lower at a given temperature between 5 and 22°C than for the reference trout (Fig. 9). What mattered, however, is that the sulting in more surplus energy being available for growth and maintaining condition in the +4°C scenario.

Discussion

Forecasting the effects of climate change on fish populations requires integration of knowledge and predictive capabilities from many levels (Shuter and Meisner 1992, Figs. 9 and 12; DeAngelis and Cushman 1990, Fig. 4). The analysis presented in this paper is one component of this broader assessment. We used simple climate change scenarios in that an annual temperature cycle was simply increased 2 and 4°C. Others have proposed that climate change could also affect the variance of temperature and cause increases in temperatures that vary with season. In addition, increases in air temperature would likely affect stream flow through changes in precipitation, snowmelt, and evapotranspiration; thus, there could be major effects on habitat availability, flood events, and periods of low flow. How

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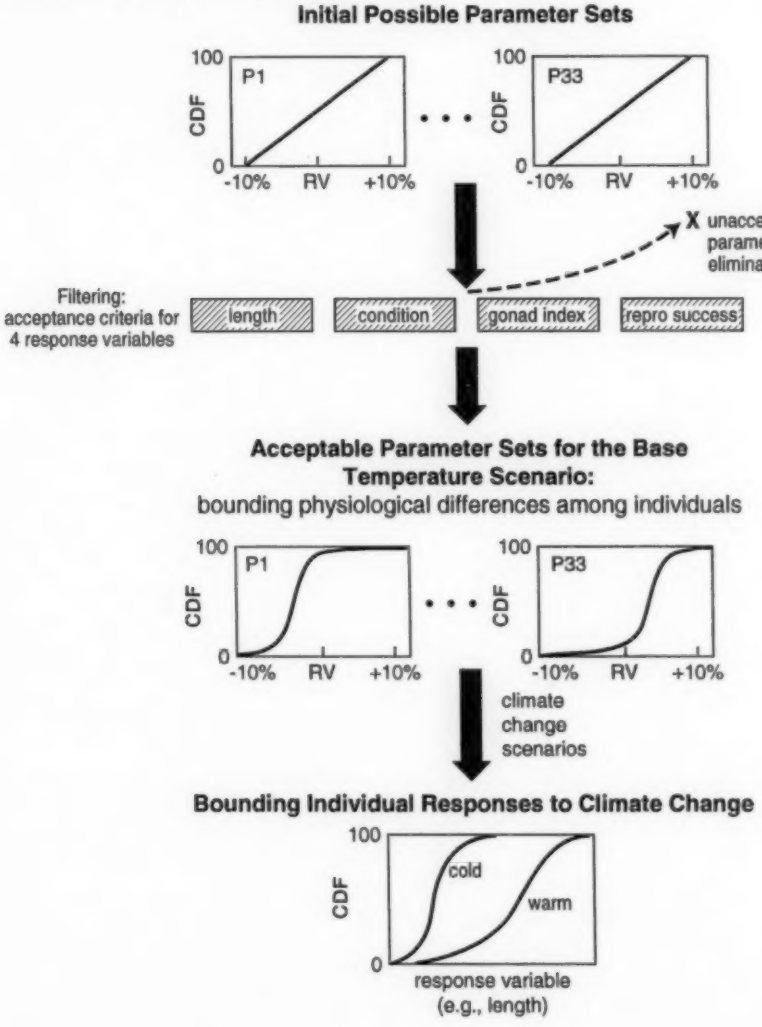
Fig. 10. Overview of steps in the climate-change impact assessment. Initially, the potential range of physiological parameter sets is defined by assuming a uniform distribution of values lying between arbitrary limits placed around the reference value (RV ± 10%, as defined by the cumulative distribution function (CDF) for the uniform distribution) for each of 33 parameters (Table 1B). Parameter sets that result in unacceptable consequences (i.e., death, reproductive failure, and unrealistic growth rates) are filtered out. The range of the remaining acceptable parameter sets establishes bounds for the differences in physiological parameters that might exist among individual members of the population under study. Bounds on the responses of individual trout to a particular climate-change scenario are then determined by evaluating the responses to the scenario across the range of acceptable parameter sets.



trout and their prey and predators would respond to changes in the seasonal patterns of temperature and flow is subject to much debate. We realize our temperature scenarios are simplistic but offer these analyses as a first step in an ongoing assessment of effects of climate change on fish populations. Rather than wait for a consensus on the details of region-specific climate change scenarios, we opted to proceed with simple scenarios. When detailed scenarios become available, more realistic model predictions of fish responses will be possible.

Model responses to these simple climate change scenarios were complicated (Fig. 7). Relative to the base temperature scenario, (*i*) at +2°C, growth decreased, condition and gonad index increased, index of reproductive success was similar, and total egg production was 96% of base; (*ii*) at +4°C, growth greatly decreased, condition and gonad index increased, IRS decreased, and total egg production was only 61% of base; and (*iii*) at –2°C, length, condition, gonad index, and IRS all decreased, and total egg production was 92% of base. These effects of the different temperature regimes on model female trout are all interpretable in terms of shifts in the balance between energy acquisition, energetic costs, and energy reallocation at various times during the annual cycle. Although the model is not currently designed for male trout, it is likely that the effects of these different temperature regimes would be

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similar but not identical. The timing of energy allocation to gonad is different for the two sexes, and activity respiration associated with reproduction is likely greater for males than females (Van Winkle et al. 1997).

The effects of a given temperature scenario varied substantially among individual model trout because of relatively small differences in their parameter values (Fig. 8). These differences may be considered analogs of genotypic differences among individuals. Small differences in parameter values (i.e., ≤6%) defining the temperature multipliers of maximum consumption and standard respiration (Fig. 9) resulted in some individuals dying with the +4°C scenario, while others grew better, were in better condition, and had a higher index of reproductive success with the +4°C scenario than the base scenario. The long-term implications of phenotypic variability in physiological parameters, in terms of selective pressure, is not readily evaluated experimentally. Combining physiological models and Monte Carlo filtering provides one approach for heuristically exploring such implications (Fig. 10), which in turn can stimulate suitable laboratory studies (Dunham and Overall 1994; Gurney et al. 1990; McCauley et al. 1990).

Our analysis involved several innovations in model structure. Five noteworthy features of the the model are (*i*) representation of four state variables (length and energy content of soma, storage, and gonad compartments); (*ii*) physiological condition affects consumption, respiration, energy allocation, mortality, sexual maturity, and spawning; (*iii*) time-varying allocation of surplus energy to soma, storage, and gonad; (*iv*) energy allocation to gonad treated as a demand function, as with respiration; and (*v*) trade-off between using surplus energy to improve physiological condition or to increase length.

Our analysis also involved innovations in parameter estimation. Biologically realistic variability in parameter values were determined by Monte Carlo filtering. Because of difficulties in specifying parameter variability a priori, differences among individuals in most individual-based models typically emerge over time from stochastic environmental effects (Chambers 1993). While each individual trout in this model is rigid in its ability to control its energy allocation, filtering permits individuals to differ in their abilities. We used filtering to estimate the amount of interindividual variability sustainable in a given environment (baseline conditions) and then used this parameter variability to project the effects of temperature changes (Fig. 10). We view these innovations in model structure and parameter variability as necessary to accurately represent the effects of temperature on how a fish allocates energy to growth and reproduction over an annual cycle.

Dynamic programming (Mangel and Clark 1988) offers an alternative approach for relaxing the rigidity imposed on individual trout in model simulations. This approach could better represent the flexibility in response that real trout possess, based on their physiological condition, prey availability, predation intensity, and abiotic variables such as temperature (Fig. 1). Our initial attempts at a dynamic programming approach were computationally intractable. Combining a variation on dynamic programming with Monte Carlo filtering may ultimately allow both individual-level decision making and genotypic variation to be simulated simultaneously. A conceptual extension of our filtering approach might involve a genetic algorithm applied at the population level over many generations to select an assemblage of genotypes.

Modeling tools for assessing climatic change effects on fish populations are available. Our model of energy allocation to growth and reproduction fills an important gap in the available suite of process and population models appropriate for forecasting environmental change (DeAngelis and Cushman 1990). This model, like all models, requires further empirical corroboration to increase our confidence in its predictions. In our opinion, however, the greatest uncertainties are not in the process and population models but in predicting realistic climate change scenarios and their effects on the magnitude and variability of temperature and flow regimes (Cushman 1991) and their effects on prey production and availability of food for trout.

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Accumulation of natural organic matter on the surfaces of living cells: implications for the interaction of toxic solutes with aquatic biota

Peter G.C. Campbell, Michael R. Twiss, and Kevin J. Wilkinson

Abstract: Reference humic substances (fulvic and humic acid) were used to demonstrate the adsorption of natural dissolved organic matter (DOM) onto the surfaces of phytoplankton (*Chlorella pyrenoidosa*, *Synechococcus leopoliensis*) and isolated fish gill cells (*Salmo salar*) at environmentally relevant pH values (4–7) and DOM concentrations (0–20 mg·L^{-1}) and various ionic strengths (0.02–0.1 M). In the presence of DOM the negative surface charge of cell surfaces increased, as monitored using the electrophoretic mobility of living cells. This effect was much greater at pH 4 than at circumneutral pH, suggesting that the interaction of DOM with cell surfaces involves either a hydrogen-bonding sorption mechanism, between electronegative functional groups present in the DOM and on the cell surface, or the formation of hydrophobic bonds between the cell surface and the hydrophobic domain of the DOM. Sorption of DOM by cell surfaces has been confirmed by direct measurements of DOM loss from solution on contact with phytoplankton cell suspensions and by the observation of the association of humic substances with phytoplankton cell surfaces using transmission electron microscopy. These results demonstrate that DOM interacts with living surfaces and may thereby influence chemical and physical processes at the cell–solution interface.

Résumé : Des substances humiques de référence (acides fulviques et humiques) ont été utilisées pour mettre en évidence l'adsorption de la matière organique dissoute naturelle (MOD) sur des surfaces biologiques. Les expériences ont été réalisées à des valeurs de pH (4–7) et à des concentrations en MOD (0–20 mg·L^{-1}) représentatives de celles rencontrées dans le milieu naturel, et à des forces ioniques entre 0,02 à 0,1 M. Comme modèles biologiques nous avons employé des cellules phytoplanctoniques (*Chlorella pyrenoidosa*, *Synechococcus leopoliensis*) ainsi que des cellules de branchies de poisson (*Salmo salar*). La charge superficielle des cellules était initialement négative; en présence de la MOD elle devenait plus négative, tel que démontré par des mesures de mobilité électrophorétique. Cet effet de la MOD était plus important à pH 4 qu'à pH 7, ce qui suggère que l'interaction de la MOD à la surface de la cellule implique soit la formation de liens hydrogène (entre des groupements fonctionnels électronégatifs présents au sein de la MOD et à la surface biologique), soit la formation de liaisons hydrophobes entre la surface cellulaire et des régions hydrophobes de la MOD. L'adsorption de la MOD à la surface des cellules a été confirmée par des mesures directes, où des pertes de MOD de la phase dissoute ont été suivies après addition de cellules phytoplanctoniques, de même que par l'observation de l'association de substances humiques aux surfaces algales à l'aide de la microscopie électronique à transmission. Les résultats de ces expériences démontrent que la MOD interagit avec des surfaces vivantes; cette interaction pourrait influer sur les processus physiques et chimiques importants qui se déroulent à l'interface cellule–solution.

Introduction

Natural dissolved organic matter (DOM) is ubiquitous in the aquatic environment, with surface-water concentrations normally ranging from 1 to 15 mg C·L^{-1} (Thurman 1985). Recalcitrant humic substances such as humic acid (HA) and fulvic acid (FA) are the major components of DOM in fresh water (generally 50–80%; Buffle 1988). They range in size from small (molecular weight <1000) to relatively large (molecular weight >100 000) macromolecules and possess a variety of largely oxygen-containing functional groups: carboxylic acid and phenolic groups dominate, with minor contributions from quinone, ketone, amine, and perhaps sulfhydryl groups (Thurman 1985).

Given their ubiquity and varied functionality, it is perhaps not surprising that humic substances have been implicated in many biogeochemical processes, e.g., metal complexation, metal redox reactions, photochemical transformations, and nutrient cycling. In Table 1, we have summarized these various roles and have indicated for each one the inherent chemical properties of DOM that lead to its involvement. In this general context (properties of DOM versus biogeochemical roles), the purpose of the present paper is to draw attention to one of the recognized properties of humic substances that has been relatively neglected by researchers in the aquatic sciences, namely the presence of both hydrophilic and hydrophobic domains within the HA macromolecule. This amphiphilic character confers surfactant properties on humic substances (Visser 1982) and favours their aggregation in solution (Wershaw 1993) and their accumulation at interfaces. The tendency of DOM to accumulate at *abiotic* surfaces was reported in the

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Table 1. Some recognized roles of natural dissolved organic matter (DOM) in the aquatic environment.

Chemical property	Biogeochemical role	Reference(s)
Acidic functional groups (carboxylic acids, R-COOH; phenols, Ø-OH)	Contribution to surface water acidity	Oliver et al. 1983
	Metal complexation	Buffle et al. 1990
	Involvement in P cycling (mixed ligand DOM–Fe–phosphate complexes)	Sunda 1994; Cotner and Heath 1990
Electron-accepting groups (quinones)	(Photo)reduction of metals (Fe, Mn)	Waite and Morel 1984; Stone and Morgan 1984
Presence of chromophores, excitable π-electrons	Photosensitized oxidation of other organic molecules	Zepp 1988; Weiner and Goldberg 1985
	Light attenuation in surface waters	Scully and Lean 1994
Presence within the macromolecule of hydrophobic structures	Adsorption/complexation of small hydrophobic organic molecules	Chiou et al. 1986
Coexistence within the macromolecule of both hydrophilic and hydrophobic domains	Accumulation of humic substances at air–water and water–particle interfaces	Hunter 1980; Tipping 1981; Davis 1982

Table 2. Elemental composition and characterization of the standard humic substances used in this study: a soil-derived FA (Armadale FA) and a water-derived HA (Suwannee River HA) (values for element composition are given as a percentage of mass).

Element	FA[a]	HA[b]
C	50.90	54.30
H	3.35	4.10
O	44.75	39.20
N	0.75	1.15
S	0.25	0.75
P	0.10	0.01
Average molecular weight (daltons)	950	1100[c]

[a]Gamble and Schnitzer (1973).
[b]Dr. P. MacCarthy, International Humic Substances Society, Colorado School of Mines, Golden, Co., personal communication.
[c]Aiken et al. (1989).

early 1980s (e.g., iron oxides, Tipping 1981; aluminum oxides, Davis 1982), but relatively little attention has been paid to the interactions of DOM at the surface of *living* cells.

In the present study, using techniques that probe the surface properties of cells and the sorption process itself, we have established that accumulation of DOM does occur at biological surfaces and that this interaction is favoured at low pH. The demonstration that DOM accumulates at biological surfaces challenges our current perceptions of the boundary between a living cell and the aquatic environment. In this paper, we consider the effects of DOM accumulation on the ambient chemical environment close to the cell membrane and speculate on the consequences of this accumulation on solute–cell interactions.

Materials and methods

Model natural organic matter solutions

The well-characterized FA (cf. Gamble and Schnitzer 1973) used in these experiments was obtained from Ecolinc Inc. (Roxboro, Que.). Prior to its commercial distribution, it had been isolated from an imperfectly drained podzol (spodosol) from Prince Edward Island, Canada, and cleaned to reduce contaminant metal concentrations. The lyophilized FA was maintained in darkness in a desiccator at room temperature until use. FA solution was dissolved in 0.01 M NaOH prior to dilution and addition to the test medium. HA (IHSS Suwannee River HA Ref. 1R101H), obtained from the International Humic Substances Society (Colorado School of Mines, Golden, Co.), was used in some experiments and manipulated in the same manner as the FA. These two humic substances have similar elemental composition (Table 2) and a lower ash content than other commercially available humic substances. The soil-derived FA was chosen to represent the predominantly allochthonous DOM present in running waters and in small water bodies in intimate contact with their watershed soils.

Although the solutions of organic acids appeared to be completely dissolved, certain problems have been reported in the literature with respect to the rehydration of lyophilized concentrates of humic substances (Perret et al. 1991). To minimize the persistence of humic substance aggregates, solutions were prepared (including initial pH adjustment using analytical-grade HCl or NaOH and filtration) and allowed to equilibrate for at least 24 h before use.

Preparation of cellular suspensions

Microorganisms

Species were chosen to represent two of the major classes of freshwater phytoplankton: the chlorophyte *Chlorella pyrenoidosa* UTCC 89 (also known as *Chlorella fusca* var. *vacuolata* UTEX 252) and the cyanobacterium *Synechococcus leopoliensis* UTEX 625 (Cyanophyta), referred to herein as *Chlorella* and *Synechococcus*, respectively. The cell surfaces of these two organisms differ in their biochemical composition. The outer cell wall surface of the *Chlorella* used here is composed of a mannose-based hemicellulose (Takeda 1988) whereas *Synechococcus*, like all cyanobacteria, possesses lipopolysaccharides imbedded in a lipid bilayer membrane at the surface of a multilamellar Gram-negative bacterial-type cell wall (Wolfe 1985).

Culture conditions were standardized for the growth and maintenance of all species. Cultures were grown in an environmental chamber with constant temperature (20°C), continuous lighting (100 μmol photons·m^{-2}·s^{-1} provided by fluorescent tubes), and gyratory shaking (60 rpm). *Chlorella* was grown in Bold's basal medium (Starr and Zeikus 1993) and *Synechococcus* in Fraquil medium (Morel et al. 1975). Cultures were transferred 1 day prior to experiments into either a modified AAP medium (AAP^-, no added trace metals or EDTA, Parent et al. 1996) or $Fraquil^-$ medium (with no added trace metals, vitamins, or EDTA). These two dilute media have similar ionic strengths and nutrient concentrations. The test media used for growth or assays with the phytoplankton did not contain organic buffers for

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pH control, since we wished to limit the organic compounds in the media to the added DOM.

Typical procedures for the initial harvesting of cells involved gentle filtration (<150 mmHg, 1 mmHg = 133.322 Pa) onto a 0.4-μm polycarbonate membrane filter (Nuclepore). Cells were rinsed on the filter using sterile culture media and resuspended in the same. Test medium was either Fraquil$^-$ or AAP$^-$; both media contained 0.01–0.1 M NaCl, added to control boundary layer thickness (see EPM experiments below).

Fish gill cells

In addition to phytoplankton, fish gill cells were also used to study the interactions of humic substances with living animal surfaces. Gill cells were isolated from a 1-kg Atlantic salmon (*Salmo salar*) by an enzymatic separation (collagenase) and differential centrifugation procedure, as described in Wilkinson et al. (1993). In brief, gill filaments were excised, washed to remove excess mucus and blood, and then digested in a collagenase medium at 30°C. Gill cells were washed and centrifuged several times in saline medium to remove cellular debris and then washed a final two times with a pH-adjusted 0.1 M NaCl solution. Microscopic examination and cell population distributions (electronic particle counter) indicated that a relatively homogenous population of cells could be harvested in this manner. Cell surface area distributions indicated that the majority of cell surface area was composed of intact cells. Experiments were done quickly (within 5 h of the preparation of the cell suspensions) and in random order to avoid artifacts that could have resulted from cell degradation over time.

Acid titration of *Chlorella*

The algae used for the acid titration experiments were grown in 2× AAP medium up to the exponential growth phase, at which point they were harvested by gentle centrifugation (1000 × *g*) and washed in AAP$^-$ medium containing no added bicarbonate. Two forward and two reverse titrations were done for both live and dead algae. For the titrations with dead cells, the algae were heat killed (60°C, 60 min); cellular integrity was verified by light microscopy. For the live algae, pH measurements were taken at a fixed time (5 min) after the addition of acid or base to control for the possible effects of uptake of acid or base by the ion channels present at the membrane surface. Titrations were performed under CO_2-free conditions: all experimental solutions were heated and then bubbled with nitrogen (12 h) before each experiment. Experiments were conducted under a N_2 atmosphere at 22°C. Upscale and downscale titrations were accomplished from pH 3.8 to pH 7.5 using high-grade NaOH and HCl, respectively.

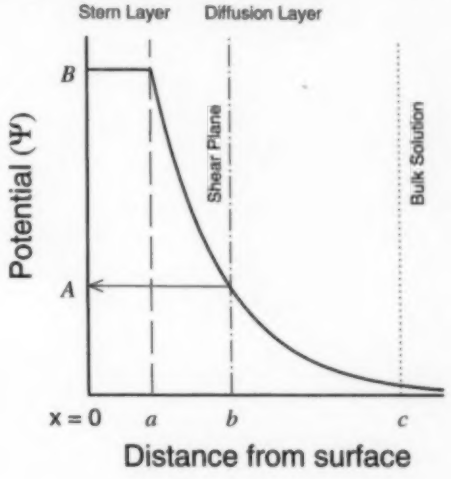
The presence of a single type of proton binding site was assumed (e.g., Stary and Kratzer 1982; Parent and Campbell 1994); nonlinear regression and a least squares optimization procedure were used to determine an overall pK_a value for the algal surface. Average values were determined by taking the mean of each of the two upscale and two downscale titration data sets.

Adsorption measurements

Batch experiments

A batch technique was used to measure adsorption of FA by phytoplankton cells. Cells in their late exponential growth phase were filtered and resuspended in solutions containing known quantities of FA. After a 100-min exposure period, cellular electrophoretic mobility (EPM) was assessed (see below) and aliquots of the cell suspensions were fixed with Lugol's iodine for cell counting. The remaining culture was filtered (0.4 μm, polycarbonate membrane), the pH of the filtrate was determined, and the FA content of the filtrate was determined by measuring its light absorbance at 400 nm. The loss of colour (A_{400}) in the filtrate, relative to the colour prior to the addition of the phytoplankton, was related to the adsorptive loss of FA to the cell surface. Light absorbance was pH corrected (if necessary) by reference to a standard curve of A_{400} versus pH at a given concentration of FA. The addition of phytoplankton alone did not affect the A_{400} value of the medium. Similarly, in the absence of phytoplankton the filtration step alone did not remove any FA (A_{400} unaffected).

Fig. 1. Diffuse double layer extending from the surface of a particle. The surface potential (*B*) is imparted to the surface by ionizable functional groups (e.g. ≡R-CO_2H, ≡R-OH, ≡R-SH, ≡R-NH_3) that are part of the surface material (e.g., protein, carbohydrate, polysaccharide). The Stern Layer (0–*a*) is the limit to which charged particles can approach the surface. The shear plane (*b*) represents the distance from the surface within which water molecules and ions associated with the surface move with the particle as it is displaced; any charged solutes outside the shear plane (*b*–*c*) will not affect the apparent surface charge (*A*) as measured by EPM. The double layer (0–*c*) is composed of the Stern Layer (0–*a*) and the diffusion layer (*a*–*c*); the shear plane ($a \leq b < c$) may shift according to the electrolyte concentration in the bulk solution. Modified from Stumm (1992).



EPM experiments

We examined the interactions of DOM with living surfaces by following changes in EPM. Interpretation of changes in the magnitude of EPM in terms of modifications of a surface charge is often tenuous, and only under relatively restricted circumstances can surface potential be calculated from values of EPM (Hunter 1981). Because EPM reflects the electric potential at the shear plane, which may not physically correspond to the potential of the Stern Layer (cf. Fig. 1), extrapolation of EPM values to surface potentials must be made with care. Nevertheless, for all experimental conditions used here (high ionic strength, large cells with resulting large radii of curvature, low measured EPM in the absence of DOM), we are clearly in the domain of validity of the Smoluchowski equation (Hunter 1981).

Surface properties of cell cultures in defined media were studied by adjusting medium acidity and (or) by adding FA. The average EPM of living cell suspensions under these conditions was measured using photon correlation spectroscopy – laser Doppler velocimetry (PCS-LDV; DELSA 440, Coulter Electronics). Relatively high ionic strength media (0.01–0.1 M) were used in all experiments to displace the shear plane towards the cell surface. As the shear plane approaches the Stern Layer, EPM becomes more directly related to the cell surface potential at the boundary of the Stern Layer and the diffusion layer (cf. Fig. 1). In addition, in high ionic strength media the

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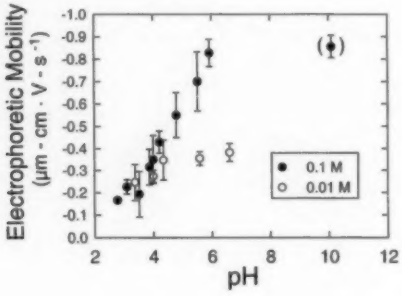
Fig. 2. Variation of the EPM of *Chlorella* as a function of pH at two ionic strengths. The measurement of EPM at pH 10.2 was not included in the regression of EPM versus pH used to estimate the pK_a of *Chlorella*. Values are means ± SD of duplicate measurements.



accumulation of charged solutes in the diffusion layer becomes relatively insignificant in comparison with the high salt concentration already present in the bulk medium. Surface potential and EPM are thus related to the species chemisorbed directly at the cell surface, i.e., within the Stern Layer. Cells were exposed to the experimental solutions for 10 min (fish gill cells) or 100 min (phytoplankton) before initiation of the EPM measurements.

Observations by transmission electron microscopy

Transmission electron microscopy (TEM) measurements were performed using a Zeiss EM 109 instrument in a procedure similar to that described by Wilkinson et al. (1995). Briefly, an aliquot of phytoplankton cells was added to a solution containing humic substances (10 mg FA·L^{-1} or 10 mg HA·L^{-1}) at pH 4.5. The cells were equilibrated for 2 h and then centrifuged directly (1000 × *g*, 1 h) onto copper (200-mesh) TEM grids covered with a small drop of hydrophilic resin (Nanoplast FB 101; Plano Electron Microscopy Suppliers, Marburg, Germany). The TEM grids were removed, the thin nanoplast layer allowed to polymerize, and the whole cells observed. No staining of the organic matter was necessary due to the presence of residual electron-dense material in the humic substances. Samples of phytoplankton at pH 4.5 with no added DOM were treated as controls.

Particle size measurements

Cell density and size were determined on samples that had been fixed with Lugol's iodine. Analysis was conducted using an electronic particle counter (Coulter Multisizer II, 15 or 30-µm orifice; Coulter Electronics) within 2 days of fixation.

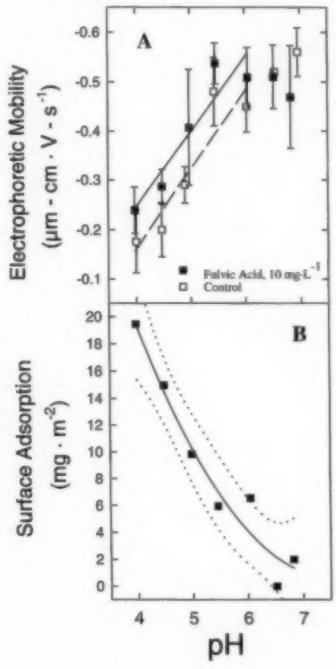
Results

Interaction of protons at the phytoplankton surface

Acid–base titrations of *Chlorella* suspensions were used to characterize the proton-binding capacity of the algal cell surface. Results for upscale and downscale titrations were similar, both for the living and nonliving cells (titration data not shown). The titration data were treated in a simplified way, in terms of a single (carboxylic acid) binding site at the algal surface (cf. Parent and Campbell 1994); this treatment yielded a pK_a of 4.2 ± 0.2 (mean ± SD, $n = 4$) for live cells and 4.0 ± 0.1 for pasteurized cells.

To obtain an independent estimate of the average pK_a value of the functional groups present at the surface of the live *Chlorella* cells, we measured the cells' EPM as a function of pH, at two different ionic strengths, and determined the intercept

Fig. 3. (A) Whole-cell EPM and (B) adsorption of FA by the cyanobacterium *Synechococcus* as a function of pH in 0.1 M NaCl. Values are means ± SD. Least squares regressions (second order): control cells EPM, $r^2 = 0.97$; FA-exposed cells EPM, $r^2 = 0.96$; 95% confidence intervals for adsorption regression.



of the EPM versus pH plots (Hunter 1981). The pK_a value of 3.9 ± 0.2 derived in this manner (Fig. 2) agrees reasonably well with the results obtained from the acid titrations.

The increase in EPM with increasing pH observed for the chlorophyte *Chlorella* (Fig. 2) was also observed for the cyanobacterium *Synechococcus* (Fig. 3A). Such trends are typically observed with algal or bacterial cells and result from deprotonation of the cell surface functional groups with increasing pH.

Effects of FA on the EPM of suspended cells

Phytoplankton cells

At a given pH, the cell surface of *Synechococcus* acquired a more negative charge in the presence of 10 mg FA·L^{-1} than in the control sample without FA (Fig. 3A). Over the pH range from 6 to 4, this difference between the FA-containing medium and the control medium was reasonably constant, even though the amount of FA associated with the algal cells increased markedly as the pH decreased (Fig. 3B). A similar independence was observed in the experiment carried out at a fixed pH (5) but with increasing FA concentrations (0–20 mg·L^{-1}; Fig. 4). The amount of FA bound to the *Synechococcus* cell surface increased as FA concentration increased (Fig. 4A), but after an initial jump between 0 and 5 mg FA·L^{-1}, EPM values remained constant (Fig. 4B).

In a similar manner to *Synechococcus*, *Chlorella* in the presence of FA also showed the anticipated decrease in EPM

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Fig. 4. (A) Whole-cell EPM of the cyanobacterium *Synechococcus* and (B) surface adsorption of FA on *Synechococcus* cells exposed to various concentrations of FA at pH 5 in 0.1 M NaCl. Cell numbers in all treatments gave the same cellular surface area (9.7×10^{-2} m^2). EPM values are means ± SD; adsorption values were obtained from single measurements. EPM and adsorption values are plotted as a function of the initial FA concentrations. Losses of FA from solution were less than 10%.

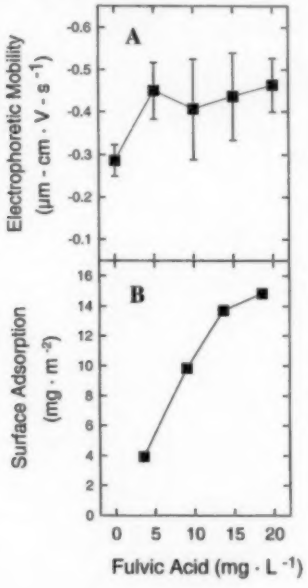


Fig. 5. Whole-cell EPM and adsorption of FA to the microalga *Chlorella* as a function of pH in AAP$^-$ medium ($I = 0.002$ M) containing 14 mg FA·L^{-1}. Least squares regressions (second order): EPM, $r^2 = 0.74$; adsorption, $r^2 = 0.99$. Data for adsorption values from Parent et al. (1996).

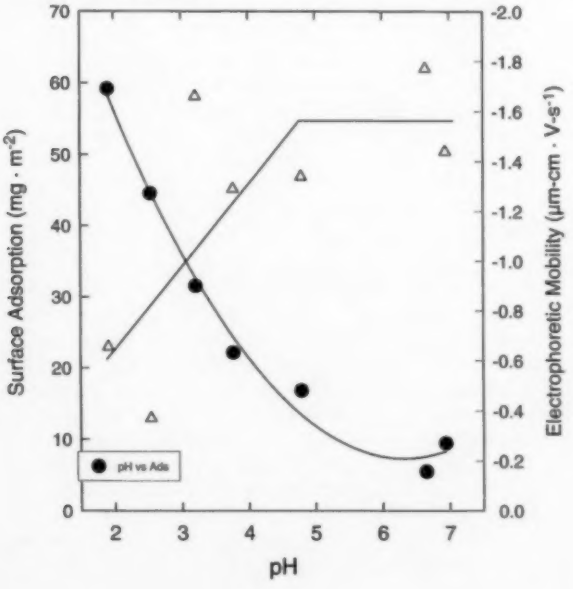


Fig. 6. EPM of isolated Atlantic salmon gill cells over a range of FA concentrations as measured at three different pH values (4, 5, 6). Values are means ± SD.

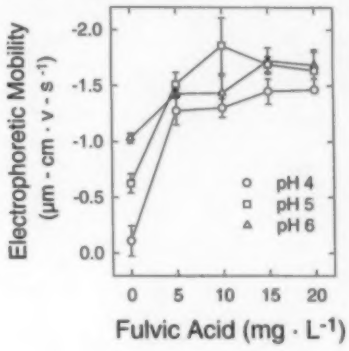
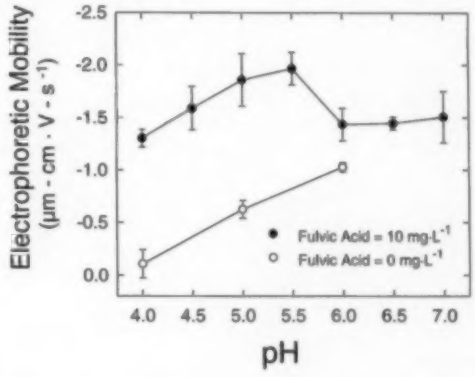


Fig. 7. EPM of isolated Atlantic salmon gill cells over an environmentally relevant pH range in the presence and absence of FA. Values are means ± SD.



with increased medium acidity, reflecting the decrease in surface potential as the surface functional groups become protonated (Fig. 5, open triangles). The decrease in pH was associated with an increase in the adsorption of FA by *Chlorella* (Fig. 5, solid circles), as had been observed for the cyanobacterial cells (Fig. 3B).

Fish gill cells

As observed with the phytoplankton, the EPM of isolated Atlantic salmon gill cells also clearly decreased as the pH decreased from 6 to 4 (Fig. 6). Addition of even the lowest concentration of FA (5 mg·L^{-1}) resulted in a marked increase in the (negative) mobility of the cells, with the greatest increase (–0.015 to –1.3 µm-cm·V-s^{-1}) being observed at pH 4. Addition of greater amounts of FA (>5 mg·L^{-1}) had little further effect on EPM values, suggesting a virtual "saturation" of the cell surface at the lowest FA treatment. For a given concentration (10 mg FA·L^{-1}), the effect of FA on gill cell mobility was markedly pH dependent (Fig. 7); the divergence between the two EPM–pH plots, indicative of DOM adsorption, occurred principally in the pH range 6.5–5. Although it is evident from the present experiments that the mobility of isolated gill cells is affected by the presence of the negatively charged

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DOM, it is unclear to what extent humic substances can be sorbed directly by an *intact* gill surface.

Quantitative estimates of DOM adsorption

The EPM results described above indicate that DOM interacts with cell surfaces. This interaction was not evaluated quantitatively with the fish gill cells because of their limited viability. For the phytoplankton cells, however, direct measurement of FA loss from solution was used to quantitate adsorption by both *Synechococcus* (Fig. 3B) and *Chlorella* (Fig. 5). In the case of *Synechococcus*, apparent saturation was observed at FA concentrations ≥20 $mg \cdot L^{-1}$ (Fig. 4A).

The similar values for FA adsorption by *Synechococcus* (Fig. 3B) and *Chlorella* (Fig. 5) suggest that the different types of cell surface represented by these two species did not affect their ability to adsorb FA. Although the FA concentrations differed in the experiments with these two phytoplankton species, the cell surface area exposed to FA was virtually identical in both experiments (*Chlorella* = 10×10^9 $\mu m^2 \cdot mg\ FA^{-1}$, *Synechococcus* = 9.7×10^9 $\mu m^2 \cdot mg\ FA^{-1}$).

TEM

The EPM and adsorption results described above demonstrate that FA interacts with the cell surface of phytoplankton and that this interaction is favoured as the pH is lowered. TEM photomicrographs of entire phytoplankton cells confirmed that FA and HA aggregates readily associated with cell surfaces, relative to controls to which no DOM had been added (*Chlorella*, Fig. 8A–8E; *Synechococcus*, Fig. 9A–9C). The association of DOM at the cell surface, as evident in the TEM photomicrographs, was clearly at a scale larger than the distance of the shear plane from the cell surface (<2 nm), estimated from surface potentials of cells under similar conditions (see below).

Discussion

DOM adsorption at the cell surface

pH dependence

In experiments on phytoplankton and fish gill cells, we have demonstrated that DOM tends to accumulate at the cell surface and that the DOM–cell interaction is favoured at low pH. This pH dependence is not surprising, given the multiple roles that H^+ activity plays in aquatic surface chemistry. For example, as the ambient pH is lowered, acidic functional groups present within the DOM macromolecule will be progressively protonated; the conformation of the individual macromolecules will change (less elongated, more compact) and their tendency to self-associate will increase. At the same time, ionization of the acidic functional groups present at the biological surface will be suppressed; electrostatic repulsion between the DOM and the surface will decrease and the possibility of hydrophobic and hydrogen bonding will thus be enhanced. The increase in DOM accumulation at the cell surface at low pH is thus consistent with either a hydrogen-bonding sorption mechanism, involving electronegative functional groups present in the DOM and on the cell surface, or the formation of hydrophobic bonds between the cell surface and the hydrophobic domain of the DOM. Our present data do not allow an unequivocal differentiation between these two possibilities, but we currently favour the hydrogen-bonding mechanism for the reasons described below.

Involvement of hydrophilic functional groups in the sorption process is understandable, since fulvic and humic substances will tend to adopt a conformation in which hydrophilic ionizable functional groups (e.g., $R\text{-}CO_2H$, R-OH, $R\text{-}NH_2$) are prevalent on the *outside* of DOM macromolecules whereas hydrophobic moieties (e.g., aliphatic chains, aromatic groups) are self-associated in the interior. The external surface of a cell in an aquatic medium is also hydrophilic, favouring diffusion of DOM through the diffusive boundary, containing loosely adhered water and counter-ions, towards the surface of the cell. Hydrogen bonding between DOM macromolecules and the cell surface, both of which have net negative charges and are hydrated, seems more plausible than a hydrophobic bonding mechanism, which would involve the thermodynamically less likely removal of water of hydration prior to contact. In addition, for hydrophobic bonding to occur, the DOM would have to undergo a major conformational change to expose the hydrophobic interior region for interaction with the cell surface. The presence of labile water molecules at the shear plane should facilitate a rapid initial association of the DOM with the cell surface. By analogy with the interaction of DOM with inorganic surfaces (Stumm 1992), the strength of DOM bonding might subsequently increase over time for larger DOM molecules as they adopt the most favourable conformation for interaction with the cell surface. Self-association between individual sorbed DOM molecules might also occur at the cell surface.

Concentration dependence

The concentration dependence of DOM adsorption at the cell surface was investigated for *Synechococcus* (EPM and direct measurement, Fig. 4) and for the *Salmo salar* gill cells (EPM, Fig. 6). In both cases the adsorption exhibited the anticipated saturation response as the FA concentration was increased, but the thresholds for apparent "saturation" were different for the two techniques.

The EPM measurements suggest that available surface sites for FA adsorption onto the cell surfaces of both the phytoplankton and the fish gill cells were saturated at bulk FA concentrations >5 $mg \cdot L^{-1}$ at pH 5 (Figs. 4B and 6). The amount of FA adsorbed by the cyanobacterium under these conditions was 4.1×10^{-6} $mol \cdot m^{-2}$ (cf. Fig. 4A; FA = 950 atomic mass units), and this FA has an estimated molecular volume of 9.8×10^{-23} m^3 at pH 5 (Schnitzer 1978). Assuming that the cyanobacterium is a perfect sphere of diameter 1.5 μm, and assuming a double-layer thickness of 2.2 nm (at $I = 0.02$, 25°C; Stumm and Morgan 1996), we calculate the available volume of the double layer to be 2.2×10^{-9} m^3. The volume of adsorbed FA is calculated to have been 2.5×10^{-9} m^3, which is approximately 10% greater than the volume of the double layer. This estimate supports the EPM measurements, which indicate that the surface was already saturated with respect to FA adsorption at a FA concentration of 5 $mg \cdot L^{-1}$, a value well within the range observed in natural waters. Support for this contention comes from the earlier work of Myers et al. (1975), who monitored the EPM of the marine diatom *Cyclotella meneghiniana* and also noted an apparent saturation response

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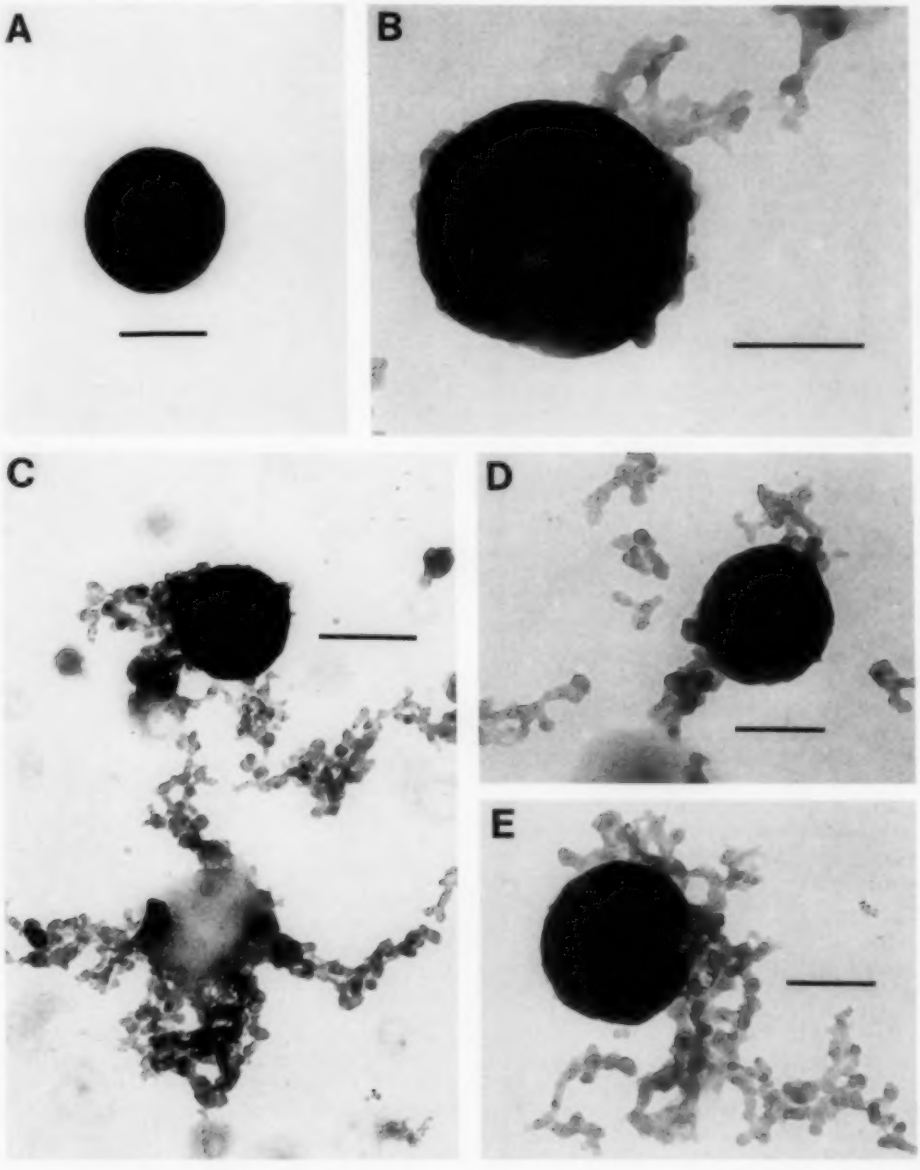
Fig. 8. TEM photomicrographs of *Chlorella* in the presence and absence of FA and HA at pH 4.5. (A) Control; (B–C) 10 mg FA·L^{-1}; (D–E) 10 mg HA·L^{-1}. Scale bar = 500 nm.



at HA concentrations >4 mg·L^{-1}, over a range of seawater salinities.

In contrast with the EPM measurements, the direct measurements of DOM adsorption at the *Synechococcus* surface at pH 5 suggest that "saturation" does not occur until the bulk FA concentration exceeds 15–20 mg·L^{-1} (Fig. 4A). Similarly, at a fixed FA concentration (10 mg·L^{-1}), the amount of FA associated with the algal cells increased markedly as the pH decreased from 7 to 4 (Fig. 3B), yet EPM of the cells did not respond to this increase in FA binding.

How can we explain this partial independence of EPM and DOM adsorption? Apparently, the negative shift in EPM reaches an upper limit upon exposure to low concentrations of FA (≤5 mg·L^{-1}: *Synechococcus*, Fig. 4A; isolated fish gill cells, Fig. 6). Maximal change in EPM would be expected to occur prior to the plateau region of the adsorption isotherm (Jones 1979). Indeed, an initial large increase in negative EPM (Fig. 4B) occurs at relatively low degrees of surface coverage (Fig. 4A), suggesting that this initial adsorption of DOM, with its conferred negative charge, markedly affects the cells' surface potential. This initial adsorption presumably occurs within the shear plane, such that the electrophoretic potential

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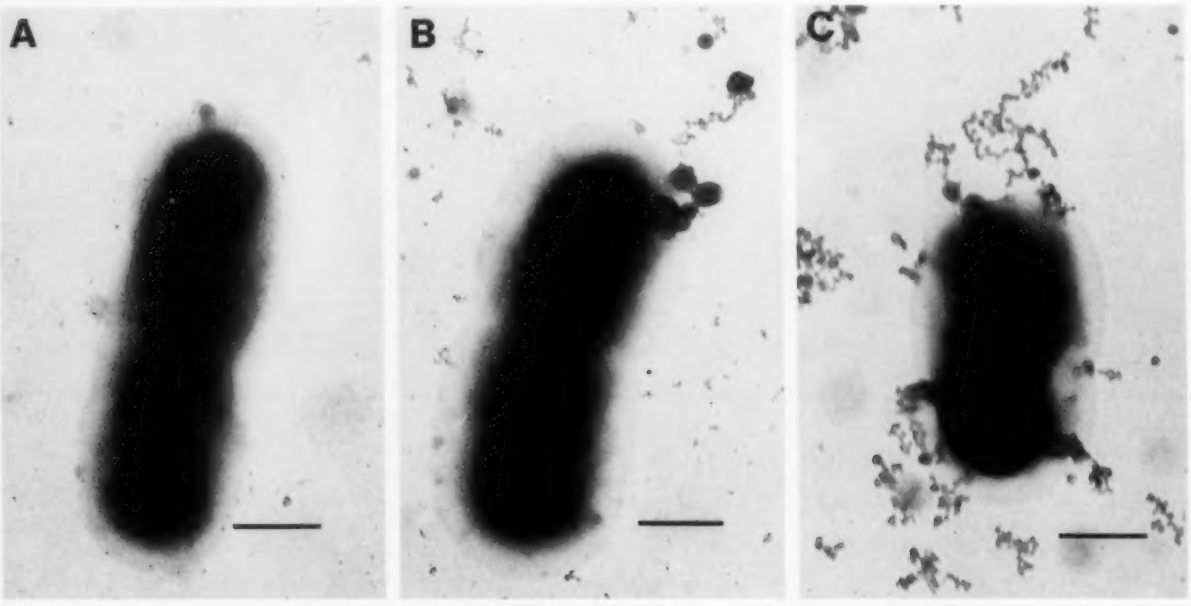
Fig. 9. TEM photomicrographs of *Synechococcus* in the presence and absence of FA and HA at pH 4.5. (A) Control; (B) 10 mg FA·L^{-1}; (C) 10 mg HA·L^{-1}. Scale bar = 500 nm.



then reflects the chemical nature of the adsorbed DOM. Since the available cell surface area is fully occupied at low DOM concentrations, any further accumulation of DOM, e.g., micelle formation on the saturated surface, would not further affect the chemical functionality or charge present at the shear plane — the EPM of the cells would thus be expected to be relatively insensitive to this additional accumulation. Indeed, according to the theoretical model described by Hesslink (1977), for relatively large particles the critical surface concentrations would be attained for 0.3 mg·m^{-2} of a branched, high molecular weight polyelectrolyte: such a level of adsorption was observed in phytoplankton exposed to FA in the present study (e.g., Figs. 3B, 4A, and 5).

Comparison with inorganic surfaces

As mentioned in the Introduction, the adsorption of natural organic matter onto *inorganic* (oxide) surfaces has been widely studied. Representative adsorption densities for DOM adsorption onto such surfaces have been compiled in Table 3. Comparison with the values observed in the present study for phytoplankton surfaces is not straightforward because of ambiguities concerning the determination of particle surface areas. Whenever possible, we have used a geometric estimation of surface area, as for our biological cells (see footnotes to Table 3 for details). Despite this difficulty in generating comparable data, DOM accumulation at biological surfaces would appear to be at least as important on an areal basis as adsorption onto inorganic surfaces, and perhaps more so. Adsorption is consistently higher at low pH, on all surfaces.

The adsorption of HA and FA at inorganic oxide surfaces is usually interpreted in terms of a ligand exchange reaction in which the carboxylic acid groups exchange for surface hydroxyl groups (Stumm 1992):

$$\equiv M\text{–}OH + R\text{–}C(O)OH \rightleftarrows \equiv M\text{–}O\text{–}C(O)\text{–}R + H_2O$$

Although this mechanism is compatible with the consistent observation of enhanced adsorption at low pH (Table 3), it is obviously untenable for a biological surface, where the surface hydroxyl groups are covalently bound to carbon and are thus not available for exchange. It is for this reason that we have suggested the involvement of a hydrogen-bonding mechanism in the association of DOM at biological surfaces:

$$\text{cell–OH} + O{=}C\begin{matrix}\diagup\text{OH}\\ \diagdown\text{R}\end{matrix} \rightleftarrows \text{cell–O- - -H- - -O–C}\begin{matrix}\diagup\text{OH}\\ \diagdown\text{R}\end{matrix}$$

DOM sorption to biological surfaces: toxicological and ecological implications

The demonstration that DOM accumulates at biological surfaces raises a number of intriguing questions about the ambient chemical environment close to the cell surface. In the following section, we consider the consequences of this accumulation on solute–cell interactions. The discussion focuses primarily on metal ions as solutes and phytoplankton as the biological surface, but some of the arguments will apply to other solutes (nutrients, organic molecules) and other surfaces.

Effects of accumulated DOM on solute behaviour close to the cell surface

The interaction of a metal with an aquatic organism will normally involve the following steps (Fig. 10): (*i*) advection or diffusion of the metal from the bulk solution to the biological surface, (*ii*) diffusion of the metal through an outer polysaccharide or glycoprotein layer (e.g., the cell wall for microorganisms or higher plants and mucus for animal cells),

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Table 3. Comparative adsorption of humic substances ($mg \cdot m^{-2}$) by various inorganic and living organic surfaces.

	Humic substance			
	HA		FA	
Surface material	pH 4	pH 7	pH 4	pH 7
γ-Al_2O_3[a]	1.25	0.70	0.84	—
α-FeOOH[b]	3.8[c]	1.9	—	—
Bentonite[d]	0.09	0.05	—	—
Kaolin[d]	0.42	0.11	—	—
Montmorillonite[d]	0.02	0.003	—	—
Quartz[d]	0.12	<0.03	—	—
Synechococcus[e,f]	27	10	20	2
Chlorella[g]	—	—	22	9

[a]Schlautman and Morgan (1994): Suwannee River HA and Suwannee River FA; values are maximum adsorption densities derived from Langmuir adsorption isotherms; $I = 0.1$ M NaCl; surface area of γ-Al_2O_3 (80 $m^2 \cdot g^{-1}$) used in this calculation was determined by 2-ethoxyethanol adsorption and is in reasonable agreement with the geometric estimation of surface area of the γ-Al_2O_3 prepared by the authors (58 $m^2 \cdot g^{-1}$).

[b]Tipping (1981): humic substances extracted from Estwaithe Water; values are maximum adsorption densities derived from Langmuir adsorption isotherms; $I = 0.002$ M NaCl–HCO_3^-; surface area of α-FeOOH used in these calculations (11 $m^2 \cdot g^{-1}$) is consistent with the surface area estimated based on the geometry of the mineral particles (19 $m^2 \cdot g^{-1}$).

[c]pH 5.

[d]Fairhurst et al. (1995): Aldrich HA; $I = 0.05$ M $NaClO_4$; values are the adsorption of HA onto surfaces for [HA] = 10 $mg \cdot L^{-1}$; all clay minerals were used at 2 $g \cdot L^{-1}$ whereas quartz was studied at a higher concentration (22.7 $mg \cdot L^{-1}$); surface areas were based on the geometric dimensions of the particles.

[e]M.R. Twiss (unpublished): Suwannee River HA; $I = 0.002$ M Fraquil growth medium; values are for adsorption at [HA] = 5 $mg \cdot L^{-1}$; surface area was based on the geometric dimensions of the cells.

[f]This study: Armadale FA; $I = 0.1$ M NaCl – Fraquil$^-$ medium; values are for adsorption at [FA] = 10 $mg \cdot L^{-1}$; surface area was based on the geometric dimensions of the cells.

[g]This study: Armadale FA; $I = 0.002$ M AAP$^-$ growth medium; values are for adsorption at [FA] = 14 $mg \cdot L^{-1}$; surface area was based on the geometric dimensions of the cells.

(*iii*) sorption/surface complexation of the metal at passive binding sites within this protective layer, or at physiologically important sites on the outer surface of the plasma membrane, and (*iv*) uptake or "internalization" of the metal (transport across the plasma membrane, cf. Simkiss and Taylor 1995).

Within this general scheme, how will the accumulation of DOM at the biological surface affect metal–cell interactions? Consider first the local DOM concentrations that are involved, taking as an example the adsorption of FA on *Chlorella*. If we assume a diffusion boundary extending 1–30 μm outward from the cell surface (dependent on cell size and relative turbulence, Tessier et al. 1994) and a surface adsorption of 12.5 mg FA·m^{-2} when exposed to a dissolved concentration of 14 mg FA·L^{-1} at pH 5 (Fig. 5), then the concentration of FA in the diffusion layer would be 30–900 times greater than that in the bulk medium (i.e., we disperse all the FA associated with the biological surface in a volume that corresponds to that of the diffusion layer surrounding the algal cell). An increase in the DOM concentration of this magnitude, close to the biological surface, would be expected to affect both metal speciation and metal diffusion. Given the known metal complexing properties of DOM, the increase in the effective ligand concentration will lead to enhanced metal complexation and an increase in the concentration of the DOM–metal complex in the diffusion layer. Similarly, the accumulation of DOM at the cell surface would tend to slow the kinetics of metal diffusion to uptake sites on the cell membrane; diffusion of cationic solutes through a matrix of fixed negatively charged functional groups is known to be slower than diffusion in normal dilute solutions (Buffle 1988), particularly if M–DOM complexes dissociate slowly.

Within the constraints of the "classical" free-ion model (FIM) of metal–organism interactions (Morel 1983; Campbell 1995), *neither* of these effects of DOM accumulation should influence metal bioavailability. According to the FIM, metal transport in solution towards the membrane and the subsequent surface complexation reaction(s) occur rapidly; the slow step is assumed to be metal internalization (step *iv*) at a transport site (X) on the cell surface. A (pseudo)equilibrium is established between metal species in the bulk solution and those at the biological surface:

$$M^{z+} + {}^-X\text{–cell} \overset{\text{fast}}{\rightleftarrows} M\text{–X–cell} \overset{\text{slow}}{\rightarrow} \text{internalized metal}$$

The biological response, whether it be metal uptake, nutrition, or toxicity, is assumed to depend on the concentration of the M–X–cell surface complex. Under such pseudo-equilibrium conditions, the concentration of the free metal ion in the microenvironment close to the cell will be identical to that in the bulk solution, $[M^{z+}]$, and the concentration of the key surface species, M–X–cell, will thus be unaffected by the presence of accumulated DOM.

A considerable body of experimental evidence has accumulated in support of the FIM. However, as we have pointed out recently (Campbell 1995; Parent et al. 1996), surprisingly few of these experiments have been carried out in the presence of natural organic matter — almost without exception, these tests of the FIM have involved the use of chemically defined media, with synthetic ligands such as ethylenediaminetetraacetic acid (EDTA) or nitriloacetic acid (NTA) as metal buffers.[3] For these conditions, the assumption of pseudo-equilibrium between metal species in the bulk solution and those at the biological surface *does* appear to be valid. However, in some cases the rate of metal internalization may be comparable with or greater than the rate of delivery to the biological surface (e.g., Fe^{3+}, see Hudson and Morel 1990). Under such conditions of kinetic control on metal availability, DOM accumulation close to the biological surface might well affect metal uptake. In other words, if pseudo-equilibrium does not prevail, i.e., if the rate-limiting step is the rate of diffusion of the metal from the bulk solution to the cell surface or the rate of reaction of the metal with the cell surface, then the presence of

[3] We know of only three successful tests of the FIM that have used natural DOM (alga, Sunda and Lewis 1978; bacterium, Sunda and Gillespie 1979; daphnid, Meador 1991), all three of which examined Cu toxicity. We are unaware of similar tests for metals other than Cu. As pointed out by W.G. Sunda (personal communication), this may be of significance, since most other biologically relevant metals, such as Zn and Fe(III), have slower ligand-exchange kinetics than Cu(II) and thus would be more likely to be subject to kinetic controls on metal uptake rates.

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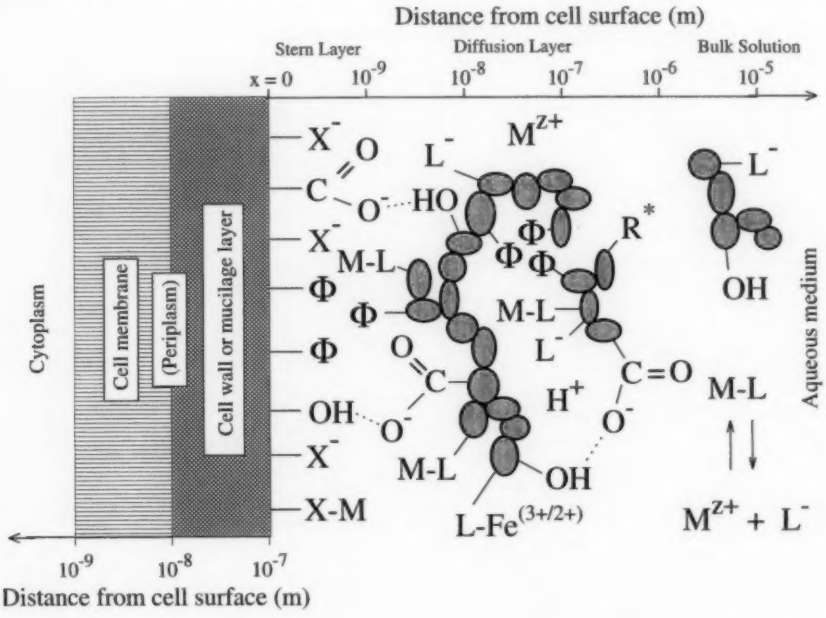
Fig. 10. Conceptual model of metal–organism interactions showing the possible role of adsorbed DOM. The periplasmic space (nm) is present between the cell membrane and cell wall in some phytoplankton cells. X^-, sensitive cell site on cell surface for trace metal internalization; Φ, hydrophobic binding site/region; L^-, trace metal complexing ligand; M^{z+}, trace metal cation; R*, excited terminal carbon atom due to photooxidation/reduction.



accumulated DOM at the cell surface will tend to retard the metal's diffusion and thus affect its bioavailability.

Effects of accumulated DOM on membrane properties

To this point in the discussion, the focus has been on solute–DOM interactions in the microenvironment close to the cell surface. Direct DOM–membrane interactions are also possible. For example, we recently presented evidence of such an effect in a study of the influence of Al and DOM on the permeability of *Chlorella pyrenoidosa* to [^{14}C]-D-sorbitol (Parent et al. 1996). As anticipated, preliminary experiments showed that exposure of *Chlorella* to inorganic Al caused a decrease in membrane permeability (cf. Haug 1984). The algal cells were then exposed at pH 5 to a constant concentration of inorganic monomeric Al but to increasing concentrations of "organic Al," i.e., the total concentrations of both Al and DOM were increased in concert, while the concentration of inorganic Al (and thus of Al^{3+}, since the pH did not vary) was kept constant. Despite this constant Al^{3+} concentration, membrane permeability progressively returned to its original state as the DOM concentration was increased; interestingly, this restoration of normal permeability corresponded to suppression of Al toxicity and restoration of normal growth.

The foregoing example suggests that the interaction of DOM with an algal surface can affect the permeability of the plasma membrane towards an uncharged but hydrophilic solute such as D-sorbitol. Since earlier work by Colman et al. (1986) showed that uptake of sorbitol by *Chlorella pyrenoidosa* takes place by passive diffusion (i.e., permeases absent), changes in the rate of sorbitol uptake can be interpreted in terms of changes in membrane integrity, as reflected by permeability.[4] Given the key role played by the plasma membrane in controlling exchanges between the cell and its environment, it is intriguing to consider whether membrane transport of other uncharged and charged solutes into and out of the cell (e.g., CO_2, HCO_3^-, NH_3, NH_4^+, PO_4^{3-}, NO_3^-, low molecular weight organic metabolites) may be affected by the accumulation of DOM at the cell surface.

Other possible consequences of DOM accumulation at the cell surface

In a more speculative vein, we considered the possibility that the elevated concentration of DOM at the cell surface might afford a certain protection against UV radiation. Such a passive "sunscreen" role has been demonstrated for the extracellular pigment scytonemin in the cyanobacterium *Chlorogloeopsis* exposed to UV-A radiation (Garcia-Pichel et al. 1992). The concentration of DOM is relatively high close to the cell surface (see above), and the conjugated aromatic functional groups present in humic substances make them efficient scavengers of photons. However, the thickness of the DOM coating adsorbed to a cell surface offers only a very short pathlength; calculations based on absorbance values measured at 310 or 370 nm, a pathlength of 30 μm, and an effective DOM concentration of 30 mg·L^{-1} indicate that the attenuation of UV irradiation due to sorbed DOM would be less than 2%.

Exposure of DOM to sunlight has recently been shown to release numerous small fatty acids (Wetzel et al. 1995) and

[4] Note, however, that the effect of the DOM was to *restore* membrane permeability; in the absence of inorganic Al, addition of the DOM did not affect the rate of sorbitol uptake.

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nitrogen-rich compounds (Bushaw et al. 1996) that are biologically available; if sorbed DOM were to react in a similar manner, this photochemical release of labile organic molecules and bioavailable nitrogen in the immediate vicinity of the cell surface might constitute a nutrient source to the organism serving as "sorbent". Note, however, that any such beneficial effect of sorbed humic substances might well be offset by the production of toxic oxygenated radicals resulting from the photooxidation of DOM in proximity to the cell membrane (Nielsen and Ekelund 1993).

A cell coating of DOM might also affect the interaction of cells with aquatic viruses. Effects might range from beneficial (the immobilization of viral particles at a distance from the true biological surface, i.e., cell membrane or cell wall) to detrimental (the enhanced scavenging of viruses by their association with the DOM adsorbed to the cell surface).

Conclusions

The capacity of DOM to influence aquatic organisms has been recognized for 40 years or more (Tipping 1990; Petersen 1991). Not surprisingly, given the heterogeneous and rather ill-defined nature of DOM, a variety of different mechanisms have been proposed to explain these effects. Both direct and indirect actions have been suggested (direct = DOM exerts a physiological effect on the organism; indirect = DOM affects the bioavailability of a limiting nutrient or a toxic solute), but in recent years, direct effects have fallen into some disrepute. As our knowledge of the roles of DOM in aquatic chemistry has expanded, the dominant tendency among researchers has been to explain the biological effects of natural organic matter in terms of its influence on the activity and bioavailability of dissolved solutes (i.e., in terms of its ability to bind and react with inorganic micronutrients and inorganic/organic toxic solutes) (cf. Morel 1983; Kukkonen et al. 1991; Sunda 1994). The demonstration in the present experiments that DOM accumulates at the surface of living cells suggests that this conceptual model may be too simple — in addition to its well-recognized roles in complexation and redox reactions *in solution* (Table 1), DOM may well exert a *direct* effect at the interface between the living organism and its environment.

We recognize that our views of DOM–organism interactions are somewhat heretical. It is hoped, however, that they will stimulate further discussion and debate on the relative importance of surface versus solution phenomena in this important research area.

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Energy sources and ecological role of crayfishes in an Ozark stream: insights from stable isotopes and gut analysis

Gregory W. Whitledge and Charles F. Rabeni

Abstract: Energy sources for the crayfishes *Orconectes luteus* and *O. punctimanus* in the Jacks Fork River, Missouri, were quantified using stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) and gut-content analysis. A dual-isotope mixing model indicated that about two thirds of crayfish production originated from allochthonous carbon sources, and 30–50% of crayfish production was derived from direct consumption of animal matter. Young-of-the-year crayfishes and adult *O. luteus* were more carnivorous than the larger adult *O. punctimanus*. Contributions of energy sources determined using the stable isotope mixing model were similar to results of gut-content analysis corrected for differential assimilation of dietary components. Proportions of crayfish production attributed to animal matter by these two methods were four to five times greater than estimates derived from uncorrected gut-content analysis. Unadjusted gut-content analysis overestimated the percentage of crayfish production from feeding directly on detritus. Production-based calculations of crayfish food consumption rates indicated that crayfishes were the dominant consumers of benthic invertebrates, detritus, and algae and may strongly influence lower trophic levels, organic matter processing, and energy flow in this system.

Résumé : Nous avons quantifié les sources d'énergie des écrevisses *Orconectes luteus* et *O. punctimanus* dans la rivière Jacks Fork (Missouri) à l'aide d'isotopes stables ($\delta^{13}C$ et $\delta^{15}N$) et de l'analyse du contenu des intestins. Un modèle mixte combinant les deux isotopes a fait ressortir que les deux tiers environ de la production des écrevisses provenaient de sources allochtones de carbone, et que 30–50 % de cette production provenait de la consommation directe de matières animales. Les jeunes de l'année et les adultes d'*O. luteus* étaient plus carnivores que les adultes plus gros d'*O. punctimanus*. Les contributions des sources d'énergie calculées à l'aide du modèle combinant les isotopes stables étaient similaires aux résultats de l'analyse du contenu des intestins, après correction pour l'assimilation différentielle des composantes alimentaires. Les proportions de la production des écrevisses attribuées à la matière animale selon ces deux méthodes étaient quatre à cinq fois plus élevées que les estimations obtenues sans correction par l'analyse du contenu des intestins. Cette analyse sans correction surestimait le pourcentage de la production provenant de l'absorption directe des détritus. Les calculs des taux de consommation de la nourriture par les écrevisses à partir de la production indiquaient que ces crustacés étaient les consommateurs dominants des invertébrés benthiques, des détritus et des algues, et pourraient avoir une influence importante sur les niveaux trophiques inférieurs, la transformation de la matière organique et le flux de l'énergie dans ce système.
[Traduit par la Rédaction]

Introduction

Crayfishes often account for a substantial proportion of invertebrate biomass and production in lakes and streams (Vannote and Ball 1972; Rabeni 1992; Momot 1995) and are an important component in the diets of many fish species in Ozark streams (Probst et al. 1984). Crayfishes are considered to be opportunistic omnivores that do not fit neatly into the concept of functional feeding groups (Hobbs 1993). They may graze on aquatic macrophytes, particularly in lentic systems, and can have a significant impact on the biomass and species composition of plant communities (Lodge and Lorman 1987; Chambers et al. 1990). Crayfishes affect benthic algal biomass and production in lentic and lotic environments (Flint and Goldman 1975; Hart 1992; Creed 1994), are important in detritus processing in some systems (Huryn and Wallace 1987), and influence the structure and biomass of benthic invertebrate communities in lakes (Hanson et al. 1990; Lodge et al. 1994). Recently, Momot (1995) suggested that crayfishes may be more carnivorous than previously thought and may be sufficiently predatory so as to potentially compete with fishes for benthic macroinvertebrates. It is apparent that crayfishes are a potentially important component of stream ecosystem dynamics, and further clarification of their ecological role is needed to understand the energetics of lotic systems.

Previous studies of crayfish diets have been based on gut-content analysis or laboratory feeding experiments. Feeding experiments may not accurately reflect crayfish diets in the presence of natural food assemblages. Gut contents are often ground beyond recognition by the gastric mill of the crayfish (Vannote and Ball 1972; Lorman and Magnuson 1978), which may result in a bias toward materials that are more resistant to digestion. Earlier studies have also not accounted for differences in assimilation efficiency among crayfish foods.

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Vascular plant detritus is often the major component of crayfish gut contents in lotic and lentic systems (Tack 1941; Prins 1968; Momot et al. 1978). However, crayfish growth in laboratory experiments is minimal on a diet composed solely of plant material but is much greater on a diet of invertebrates or a mixed plant and animal diet (Hill et al. 1993).

Stable isotope analysis is an alternative technique for identifying carbon sources and has been used to elucidate major energy pathways in estuarine and marine systems (Fry and Sherr 1984; Peterson et al. 1985), lakes (Rau 1980; Kling et al. 1992), and rivers (Rounick et al. 1982; Rosenfeld and Roff 1992; Junger and Planas 1994). Carbon and nitrogen both have more than one stable isotope (^{13}C and ^{12}C; ^{15}N and ^{14}N) and are the elements most often used in food web studies. Changes in the ratio of heavy to light isotopes (fractionation) between biological and nonbiological materials often lead to distinct isotopic compositions among different types of primary producers (Ehleringer et al. 1986). Stable isotope ratios of consumers reflect the isotope ratios of all food sources assimilated, integrated over the period of time required for elemental turnover in tissues (Anderson et al. 1987; Kling et al. 1992). Because the $^{13}C/^{12}C$ ratio changes very little with increasing trophic level (DeNiro and Epstein 1978; Fry and Sherr 1984), stable carbon isotope analysis is useful for indicating which primary producers are ultimately important sources of nutrition for consumers. The $^{15}N/^{14}N$ ratio of an animal also reflects the $^{15}N/^{14}N$ ratio of its food sources, although consumers tend to assimilate ^{15}N from their diet preferentially over ^{14}N (DeNiro and Epstein 1981). This enrichment in ^{15}N tends to be consistent with increasing trophic level (Minagawa and Wada 1984). Thus, the $^{15}N/^{14}N$ ratio of a consumer is dependent on the $^{15}N/^{14}N$ ratios of primary producers from which it ultimately derives its nitrogen and the trophic level of the organism (Kline et al. 1993). Concurrent use of stable carbon and nitrogen isotopes in food web studies increases the probability of identifying ultimate carbon sources of organisms and allows trophic relations to be evaluated (Peterson et al. 1985; Hobson and Welch 1992).

The objectives of this study were to quantify the trophic base and assess the ecological role of the crayfishes *Orconectes luteus* and *O. punctimanus* in the Jacks Fork River, Missouri, during the growing season using stable carbon and nitrogen isotopes and gut-content analysis. Most stable isotope studies of freshwater lotic food webs to date have been qualitative, and none have focused on crayfishes. Combining stable isotope and gut analyses is often advantageous (Harrigan et al. 1989; Mihuc and Toetz 1994), and we have used both a dual-isotope mixing model and a bioenergetics model to quantify the contributions of various carbon sources to crayfish production.

Methods

Study area

The Jacks Fork River in south-central Missouri has a mean annual discharge of 12.5 $m^3 \cdot s^{-1}$ and is typical of Ozark streams. It is heavily spring fed with a medium gradient (≈1 $m \cdot km^{-1}$) and well-developed riffle and pool sequences. Stream width is typically 5–25 m, and pools >2 m deep are common. The substrate is predominantly cherty dolomitic limestone, and gravel–cobble particle size is dominant. Periodic spates and overall fast current velocities account for the typical erosional substrate. The deposition of fine materials is limited to side areas, backwaters, and deep pools. On the stream margins are numerous beds of water willow (*Justicia americana*), an emergent macrophyte that grows in colonies and is especially common in the vicinity of riffles (Rabeni 1992). The study was carried out in a fourth-order stream reach.

Crayfish collections

Crayfishes were collected in June and October 1994, which correspond to 2–4 weeks after the first appearance of free-living young-of-the-year (YOY) and the end of the annual growing season, respectively (Muck 1996). Each month, YOY and adults of each species were collected using a seine, quadrat sampler, or dip net. Crayfishes were classified as either YOY or adults based on carapace lengths (Muck 1996). Crayfishes were placed in freezer bags, frozen with dry ice or liquid nitrogen within minutes of capture to halt digestion, and kept frozen in the laboratory (–10°C) until gut-content analysis was conducted.

Gut-content analysis

Four-hundred fifty-five crayfishes from the two collecting periods were dissected, and their foreguts removed. Only contents of the foregut were examined, because nearly all items in the mid- and hindguts were digested beyond recognition. The foregut of each crayfish was split open, the contents placed in a Petri dish containing a small amount of water, distributed as evenly as possible, and viewed under a dissecting scope at 30×. Gut contents were categorized as filamentous algae, diatoms, terrestrial plant detritus, or animal. The percentage of the total area of the dish covered by each food category was estimated with the aid of a 5-mm^2 grid, which was laid under the dish containing the ingested materials. Subsamples of gut contents from selected crayfish were viewed at higher magnification to confirm the identity of food items. All foods of animal origin were identified to the fullest extent practicable. Crayfishes were refrozen immediately after dissection.

Stable isotope analysis of crayfishes and their foods

Stream-conditioned leaves from terrestrial vegetation, *Cladophora*, epilithic diatoms, heptageniid mayflies, and non-tanypodinine chironomids were collected from the Jacks Fork River for stable isotope analysis during the months in which crayfishes were sampled. Invertebrates (10–20 individuals per sample) and plants were collected from multiple habitats during each month to account for spatial and temporal variation in their isotopic compositions (Boon and Bunn 1994). All plant and invertebrate samples were placed on ice and frozen (–10°C) upon return to the laboratory. Algae were acidified with 1 M HCl to remove inorganic carbonates. Stable isotope analysis was also conducted on abdominal muscle tissue from 10–15 individuals of both YOY and adult *O. luteus* and *O. punctimanus* each month. All samples were lyophilized and homogenized by grinding into a fine powder. Isotopic analysis was performed at Coastal Science Laboratories in Austin, Tex., using a Micromass SIRA series II isotope-ratio mass spectrometer. Isotope ratios are reported in standard delta (δ) notation, which is defined as the parts per thousand deviation from the standard reference materials (air for N and Pee Dee belemnite carbonate for C) and is given by

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where R represents $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Peterson and Fry 1987). Samples with negative values are depleted in the heavier isotope relative to the standard, while those with positive values are enriched in the heavier isotope relative to the standard (Peterson and Fry 1987). The reproducibility of these measurements is ±0.2‰ for $\delta^{13}C$ and ±0.3‰ for $\delta^{15}N$ (K. Winters, Coastal Science Laboratories, Austin, Tex., personal communication).

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Stable isotope model of crayfish energy sources

A stable isotope mixing model was used to quantify the relative contributions of energy sources to crayfish production in the Jacks Fork River. This model was based on the fact that the isotopic signature ($\delta^{13}C$ and $\delta^{15}N$) of an organism reflects the relative proportions of food sources assimilated. In a study using two stable isotopes, the contributions of a maximum of three isotopically distinct energy sources can be determined (Harrigan et al. 1989). In the Jacks Fork River, there were five major energy sources potentially available to crayfishes: allochthonous (terrestrial) detritus, diatoms, filamentous algae, water willow, and aquatic insects. There were no significant differences in either $\delta^{13}C$ or $\delta^{15}N$ of any potential food type between June and October (t-test, $p > 0.15$ for each food type), so data from the two sampling periods were combined. We eliminated water willow as an energy source because it is a coarse, woody, emergent macrophyte that is probably of low nutritional value (atomic C:N ratio ≈ 40:1), crayfishes did not consume leaves or stems of live water willow in laboratory experiments, and most crayfishes in the Jacks Fork River were too small to consumptively or nonconsumptively destroy water willow (Whitledge 1996). To further reduce the number of food sources to the required three, the contribution of diatoms to crayfish production was fixed at 3% prior to use of the model based on their consistently low abundance (1–4% of total volume) in gut contents. Mean $\delta^{13}C$ and $\delta^{15}N$ measurements of the three remaining sources (terrestrial plant detritus, filamentous algae, and aquatic insects) were corrected for trophic enrichment by adding 0.8‰ to $\delta^{13}C$ (DeNiro and Epstein 1978; Junger and Planas 1994) and 3.4‰ to $\delta^{15}N$ (Minagawa and Wada 1984). The $\delta^{13}C$ and $\delta^{15}N$ values for aquatic insects were assigned using a weighted mean of 60% Chironomidae (mean $\delta^{13}C = -28.3$‰, mean $\delta^{15}N = 5.2$‰) and 40% Ephemeroptera (mean $\delta^{13}C = -29.7$‰, mean $\delta^{15}N = 4.6$‰) based on the relative proportions of these taxa in crayfish gut contents. The $\delta^{13}C$ and $\delta^{15}N$ values of each food source corrected for trophic enrichment represent isotopic signatures that crayfishes would have if they consumed only that food source. Contributions of terrestrial plant detritus, filamentous algae, and aquatic insects to crayfishes were estimated graphically by comparing isotopic signatures of each of these energy sources to $\delta^{13}C$ and $\delta^{15}N$ values of each species and age-class of crayfish averaged over the June and October sampling periods and were calculated by

$$\%\ X \text{ in diet} \times \left(\frac{(1/DA + 1/DB + 1/DC) - 1/DX)}{(1/DA + 1/DB + 1/DC)} \right) \times 100$$

where DA, DB, and DC are lengths of lines connecting mean isotopic signatures of crayfishes to isotopic signatures of each energy source corrected for trophic enrichment and X is source A, B, or C. The line lengths were determined using $\delta^{13}C$ and $\delta^{15}N$ values as Cartesian points (Kline et al. 1993).

To quantitatively assess the carbon base of crayfishes in the Jacks Fork River, the contribution of aquatic insects to crayfishes calculated using the stable isotope mixing model was apportioned into fractions derived from allochthonous and autochthonous energy sources. Quantitative determination of the trophic base of chironomids and mayflies was based on the above mixing equation using mean $\delta^{13}C$ and $\delta^{15}N$ values for these two taxa and isotopic signatures of terrestrial plant detritus, diatoms, and filamentous algae as energy sources corrected for trophic enrichment. Weighted mean isotopic compositions of 60% chironomids and 40% mayflies, based on microscopic analysis of gut contents, were used to assess the relative proportions of these three carbon sources passing through aquatic insects to crayfishes. The portion of crayfish production attributed to aquatic insects was then partitioned into allochthonous and autochthonous components. Finally, the percentages of crayfish production derived from terrestrial plant detritus and algal sources through direct and indirect consumption were added to determine the relative contributions of allochthonous and autochthonous organic matter to crayfishes.

Production-based model of crayfish energy sources and food consumption rates

Results of gut-content analyses were coupled with crayfish production estimates (Rabeni 1992) in a simple bioenergetics model (Benke and Wallace 1980) to provide a second method for quantifying the trophic base of crayfishes and to assess their ecological role in the Jacks Fork River. Use of this bioenergetics model to calculate the proportion of crayfish production derived from various food types and the amount of each food type ingested and egested annually required information on annual production of crayfishes, mean percentages of food types ingested, and assimilation and net production efficiencies for each food category.

Dry mass production estimates for YOY and adult *O. luteus* and *O. punctimanus* were obtained from Rabeni (1992). Mean proportions of food types ingested by each species and age-class of crayfish were determined from gut-content analysis of all individuals collected in June and October. A net production efficiency (NPE) of 50% was assumed for each food category (Benke and Wallace 1980). Assimilation efficiencies (AE) for *O. luteus* were measured in the laboratory using a gravimetric technique. Foods chosen for determination of AE were chironomids (*Chironomus*), *Cladophora* sp., and stream-conditioned American elm leaves (*Ulmus americana*). Diatoms were assigned the same AE as filamentous algae. All experiments were performed at 16–17°C. Crayfish were starved for 48 h prior to each experiment to clear their digestive tracts, placed in separate 2-L feeding chambers, and given a ration equivalent to about 10% of their dry body weight. Each crayfish was allowed to feed for 2–3 h or until all food was consumed and then transferred to another 2-L chamber where fecal pellets were collected for the next 24 h. Fecal pellets and any uneaten portions of the ration fed to each crayfish were dried at 60°C for 48 h and weighed to the nearest 0.0001 g. No attempts were made to correct for weight losses due to leaching or microbial activity. Dry weight of food consumed by each crayfish was determined from the difference between the dry weights of rations and uneaten portions of food. AE was calculated using the following equation: AE (%) = [1 – (fecal pellet dry wt. ÷ dry wt. of food consumed)] × 100. The AE of each food type was calculated as the mean AE for all crayfish fed that food type. The AE values for *O. luteus* were assumed to be the same for *O. punctimanus*. Annual production estimates, mean proportions of food types ingested, and AE and NPE for each food type were then used to calculate the amount of crayfish production derived from each food type and the amounts of each food type ingested and egested annually by crayfishes. See Benke and Wallace (1980) for computational details.

Results

Gut content analysis

Terrestrial plant detritus was the most abundant and frequently consumed food type for both YOY and adults of each species and constituted more than 75% of the total volume of foregut contents (Fig. 1). Filamentous algae (mainly *Cladophora* sp.) and diatoms were also consumed, although generally in much smaller amounts. Mean proportions of food types in crayfish diets averaged over the 2 months sampled ranged from 5 to 11% for animal matter, from 79 to 88% for detritus, from 1 to 2% for diatoms, and from 4 to 8% for filamentous algae (Fig. 1). Very little seasonal variation was observed in the proportions of food types in crayfish gut contents. The mean proportion of animal matter in the diet of adult *O. punctimanus* was <5%, while the mean proportion of animal matter in the diets of adult *O. luteus*, YOY *O. luteus*, and YOY *O. punctimanus* was 11% in all cases (Fig. 1).

Approximately 85% of food items classified as animal

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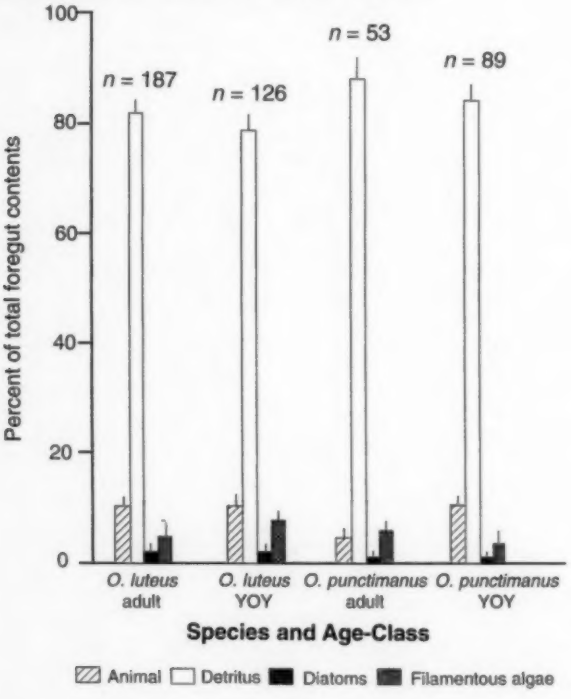
Fig. 1. Mean proportions of food types (by area) in foregut contents of each species and age class of crayfish + 1 SE. YOY, Young-of-the-year; *n*, number of crayfish examined.



matter were identifiable to some taxonomic level. These items ($n = 236$) comprised primarily chironomids (60.8%) and mayflies (mainly Heptageniidae) (29.3%). Most chironomids consumed (99%) were from subfamilies other than Tanypodinae. During the 2 months sampled, cannibalism was infrequent (crayfishes constituted 4.3% by number) and other items (Plecoptera, Trichoptera, fish, Hydracarina, Oligochaeta, terrestrial insects, Megaloptera, and Psephenidae) made up only 5.6% of all animal matter consumed.

Stable isotope ratios of crayfishes and primary producers

The three major vegetative carbon sources available to crayfishes in the Jacks Fork River, allochthonous detritus, filamentous algae, and diatoms, had non-overlapping ranges of isotopic signatures and were distinguishable on the basis of the dual stable isotope analyses (Fig. 2). Mean $\delta^{13}C$ values of all species and age-classes of crayfish ranged from –27.4‰ to –28.4‰ (Fig. 2), and were within the range of terrestrial plant detritus $\delta^{13}C$ values. Mean $\delta^{15}N$ values of adult *O. luteus* and YOY crayfishes were enriched by about 1‰ relative to adult *O. punctimanus* (Fig. 2).

Crayfish energy sources

After correction of gut-content results for differential assimilation of dietary components using AE calculated for each food type (Table 1), the percentage of crayfish production attributed to animal matter ranged from 23 ± 8.5% (mean ± SE) for adult *O. punctimanus* to about 40% for YOY crayfishes and adult *O. luteus* (Table 2). Consumption of diatoms accounted for

Fig. 2. Dual-isotope plot of primary producers and crayfishes from the Jacks Fork River. Points represent mean isotopic signatures of primary producers, non-tanypodinine chironomids, heptageniid mayflies, and crayfishes ± 1 SE (without corrections for trophic enrichment). Boxes correspond to observed ranges of $\delta^{13}C$ and $\delta^{15}N$ for each primary producer. YOY, Young-of-the-year; *n*, number of samples analyzed.

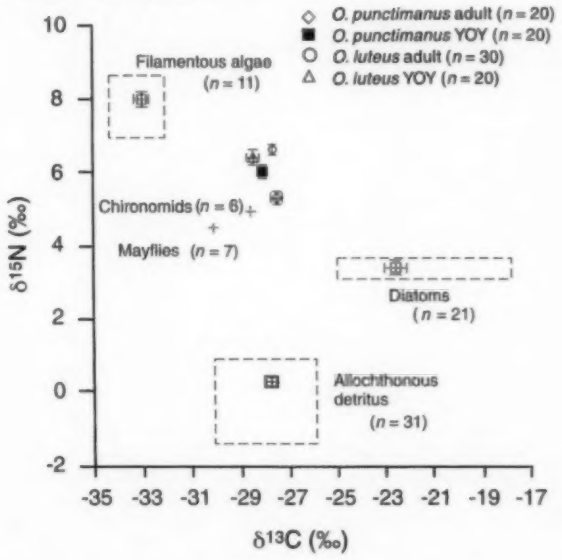


Table 1. Mean assimilation efficiencies, with SE given in parentheses, of three food types for *Orconectes luteus*.

Food type	*n**	Mean assimilation efficiency (%)
Chironomids (*Chironomus riparius*)	7	92 (1.07)
Filamentous algae (*Cladophora*)	6	39 (2.86)
Detritus (conditioned elm leaves)	6	14 (3.92)

*Number of crayfish in each experiment.

about 2–3% of production for all species and age-classes, while approximately 7–13% of crayfish production was derived from filamentous algae. The percentage of crayfish production attributed to feeding on terrestrial plant detritus was 63 ± 8.0% for adult *O. punctimanus* and approximately 44–49% for all other crayfishes.

Results of the stable isotope mixing model indicated that 29 ± 5.6% of production of adult *O. punctimanus* was derived from animal matter, with 56 ± 7.0% coming from feeding on terrestrial plant detritus (Table 2). Forty-three to 51% of production of adult *O. luteus* and YOY crayfishes was attributed to animal matter by the model, while 32–40% of production of these crayfishes was ascribed to consumption of terrestrial plant detritus. Filamentous algae accounted for about 11–15% of production of all species and age-classes of crayfish according to the mixing model.

Non-tanypodinine chironomids and heptageniid mayflies depended primarily on allochthonous detritus as a carbon source (Table 3). Approximately 60% of their energy was attributed to terrestrial plant detritus, with about 20% drawn from diatoms and another 20% obtained from filamentous

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Table 2. Contributions of energy sources to crayfishes determined by gut-content analysis, gut-content analysis corrected for differential assimilation of food types, and the stable isotope mixing model.

	Animal	Detritus	Diatoms	Filamentous algae
Gut-content analysis (uncorrected)				
O. luteus adult	11 (1.4)	82 (1.7)	2 (0.4)	5 (0.6)
O. luteus YOY	11 (1.8)	79 (2.3)	2 (0.4)	8 (0.6)
O. punctimanus adult	5 (2.1)	88 (2.6)	1 (0.8)	6 (0.5)
O. punctimanus YOY	11 (1.9)	84 (2.7)	1 (0.4)	4 (0.7)
Gut-content analysis (corrected)				
O. luteus adult	42 (5.0)	47 (4.2)	3 (1.3)	8 (2.6)
O. luteus YOY	40 (5.2)	44 (4.7)	3 (1.1)	13 (2.3)
O. punctimanus adult	23 (8.5)	63 (8.0)	2 (1.7)	12 (2.3)
O. punctimanus YOY	42 (5.9)	49 (4.6)	2 (1.1)	7 (2.1)
Stable isotope mixing model				
O. luteus adult	51 (4.6)	32 (4.5)	3	14 (0.2)
O. luteus YOY	49 (8.8)	32 (9.1)	3	15 (0.3)
O. punctimanus adult	29 (5.6)	56 (7.0)	3	11 (1.4)
O. punctimanus YOY	43 (4.8)	40 (5.6)	3	14 (0.6)

Note: Values are mean percentages of crayfish production derived from each energy source, with SE given in parentheses. YOY, Young-of-the-year.

Table 3. Trophic base of heptageniid mayflies and non-tanypodinine chironomids determined using the stable isotope mixing model.

	Terrestrial detritus	Diatoms	Filamentous algae
Ephemeroptera	57 (3.4)	20 (1.8)	23 (3.7)
Chironomidae	63 (5.4)	19 (2.2)	18 (3.2)

Note: Values are percentages of insect production derived from each carbon source, with SE given in parentheses.

Table 4. Allochthonous and autochthonous organic matter contributions to crayfishes in the Jacks Fork River determined using stable isotopes.

	Allochthonous	Autochthonous
O. *luteus* adult	63 (49–77)	37 (23–51)
O. luteus YOY	62 (39–85)	38 (15–61)
O. punctimanus adult	74 (57–91)	26 (9–43)
O. punctimanus YOY	66 (50–82)	34 (18–50)

Note: Values are percentages of crayfish production derived from each source, with the 95% confidence interval given in parentheses. YOY, Young-of-the-year.

algae. Partitioning the proportion of crayfish production derived from aquatic insects into allochthonous and autochthonous components and then adding direct and indirect contributions of terrestrial plant detritus and algal sources to crayfishes revealed that approximately 50–80% of crayfish production in the Jacks Fork River originated from allochthonous organic matter (Table 4).

Annual rates of food consumption and egestion

Crayfishes consumed a total of approximately 82.24 g organic matter·m^{-2}·$year^{-1}$, of which 7.83 g·m^{-2}·$year^{-1}$ was animal matter, 66.43 g·m^{-2}·$year^{-1}$ was terrestrial plant detritus, 1.22 g·m^{-2}·$year^{-1}$ was diatoms, and 4.76 g·m^{-2}·$year^{-1}$ was filamentous algae (Table 5). Adult *O. punctimanus* consumed about one half to one third of the amount of animal matter consumed by YOY crayfishes or adult *O. luteus*. YOY *O. punctimanus* had the highest rate of animal matter consumption and consumed more organic matter per year than adult crayfishes or YOY *O. luteus*. Approximately 77% (63.12 g·m^{-2}·$year^{-1}$) of the total amount of organic matter ingested annually by crayfishes was egested as fine particulate organic matter (FPOM), of which 93% (56.84 g·m^{-2}·$year^{-1}$) was derived from terrestrial plant detritus.

Discussion

Gut-content analysis

Proportions of food types in crayfish gut contents were similar to those of many other stream- and lake-dwelling crayfishes, which also consumed large amounts of vascular plant detritus and lesser amounts of algae and animal matter (Tack 1941; Prins 1968; Momot et al. 1978; True 1990).

Greater consumption of animal matter by YOY crayfishes and adult *O. luteus* compared with adult *O. punctimanus* may be related to the necessity for protein-rich food sources to support rapid growth of YOY crayfishes and adult *O. luteus*, which are more susceptible to fish predation than the larger adult *O. punctimanus* (Rabeni 1992). Large crayfishes have slower growth rates and need less dietary protein but have increased energy requirements compared with smaller crayfishes (Lorman 1975), which may account for the consumption of less animal matter and more detritus by adult *O. punctimanus* than the smaller adult *O. luteus* and YOY of both species. Adult *O. punctimanus* may also be more detritivorous because their large size relative to prey may make them less effective at capturing small invertebrates within the substrata.

Chironomids typically constitute from 10 to 40% of the total numbers of benthic invertebrates in the Jacks Fork River during summer and fall (C.F. Rabeni, unpublished data) but accounted for 61% of the total number of animal matter items consumed by crayfishes. This suggests that crayfishes may be selectively feeding on chironomids, perhaps because they are relatively slow moving and soft bodied, making them easy to

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Table 5. Annual rates ($g \cdot m^{-2} \cdot year^{-1}$) of production, consumption of various food types, and egestion for crayfishes in the Jacks Fork River.

	Production	Amount of food type consumed	Egested as detritus
O. *luteus* adult	2.07		
Animal		1.89	0.15
Detritus		13.90	11.95
Diatoms		0.32	0.19
Filamentous algae		0.85	0.52
O. luteus YOY	2.44		
Animal		2.12	0.17
Detritus		15.34	13.19
Diatoms		0.38	0.23
Filamentous algae		1.63	0.99
O. punctimanus adult	1.92		
Animal		0.96	0.08
Detritus		17.28	14.86
Diatoms		0.20	0.12
Filamentous algae		1.18	0.72
O. punctimanus YOY	3.13		
Animal		2.86	0.23
Detritus		21.91	18.84
Diatoms		0.32	0.20
Filamentous algae		1.12	0.69
Totals	9.56	82.24	63.12

Note: Production values are from Rabeni (1992). YOY, Young-of-the-year.

capture and digest. The low incidence of crayfish remains in foregut contents of crayfishes from the Jacks Fork River indicated that cannibalism was uncommon, even though very small YOY crayfishes would seem to be highly vulnerable to predation by larger crayfishes during early summer. Crayfishes will also consume their own exuviae (Lodge and Hill 1994), which may account for exoskeleton fragments found in some gut contents.

Crayfish energy sources

After correction of gut-content analyses for differential assimilation of dietary components, the percentage of crayfish production attributed to animal matter was four to five times greater for each species and age-class of crayfish compared with uncorrected gut-content analysis. Adjustments to gut-content analysis reduced estimates of the contribution of terrestrial plant detritus to crayfish production by 23–35% and increased the proportions of crayfish production attributed to filamentous algae and diatoms by 3–6% and 1–2%, respectively. The relatively small differences between the percentages of crayfish production attributed to filamentous algae and diatoms by corrected and uncorrected gut-content analyses were because they constituted a relatively small proportion of crayfish diets in the Jacks Fork River and were not as easily assimilated as animal matter.

Contributions of energy sources to crayfishes determined using the stable isotope mixing model were similar to results of gut-content analysis corrected for differential assimilation of food types. The two methods generally agreed within 2 SEs for each food category, suggesting that both techniques can provide reliable quantitative estimates of the contributions of energy sources to production of crayfishes and other aquatic organisms. Each method offers some advantages with respect to the other. Stable isotope analysis does not require estimates of AE or NPE, integrates resource use over longer time periods, and is less tedious, particularly when working with small organisms. Correcting gut-content analysis with a simple bioenergetics model is cheaper and is the only appropriate technique if energy sources are not isotopically distinct. Gut-content analysis can also provide information about consumption of specific taxa and may be useful for eliminating some potential foods from consideration before construction of an isotopic mixing model. Both methods reflect organic matter assimilated and more realistically estimated the relative contributions of animal matter and allochthonous detritus to crayfish production in the Jacks Fork River than uncorrected gut-content analysis. Our findings agree with Momot's (1995) hypothesis that crayfishes are more carnivorous than previously thought and that the nutritional importance of plant detritus to crayfish growth has been exaggerated.

A substantial portion of the percentage of crayfish production ascribed to feeding on terrestrial plant detritus may be derived from microbes associated with detritus rather than the detritus itself. Although microbial biomass on decaying leaves is typically quite small (Bowen 1987), microbes are easily digested (Hargrave 1970) and could contribute significantly to crayfish production given the large amount of detritus and associated microbes ingested annually by crayfishes.

Approximately one half to three fourths of crayfish production in the Jacks Fork River originates from allochthonous organic matter, which is somewhat surprising in that crayfishes are known to feed extensively on algae in other systems (Flint and Goldman 1975; Hart 1992; Creed 1994), and the study area was in a fourth-order stream reach exposed to direct sunlight several hours a day. That crayfishes might preferentially feed on detritus seems unlikely given its low nutritional quality. The Jacks Fork River flows through a largely forested watershed, and an abundance of woody debris, water willow beds, and cobble substrate in the stream channel act as debris dams, thus retarding downstream transport of coarse particulate organic matter (CPOM; Bilby and Likens 1980). Factors negatively influencing availability of benthic algae to crayfishes may include nutrient limitation of algae and frequent scouring spates, both of which have been implicated in the limitation of algal biomass in northern Ozark streams (Lohman et al. 1992). Stonerollers (*Campostoma* spp.) can also significantly reduce algal biomass in streams (Power and Matthews 1983), and the large stoneroller population in the Jacks Fork River could be monopolizing the available benthic algal resource.

Trophic role of crayfishes

Crayfishes in the Jacks Fork River play multiple functional roles and have the potential to significantly impact the biomass and production of lower trophic levels (Fig. 3). The 82 g organic $matter \cdot m^{-2} \cdot year^{-1}$ consumed by crayfishes in this system is somewhat less than the rate of organic matter consumption by crayfishes in some lentic systems (Hessen and Skurdal 1988; Saffran and Barton 1993) but is nearly twice the amount estimated to be consumed by the remainder of the benthic invertebrate community in the Jacks Fork River (Rabeni et al. 1994). Crayfishes clearly play an important role as predators

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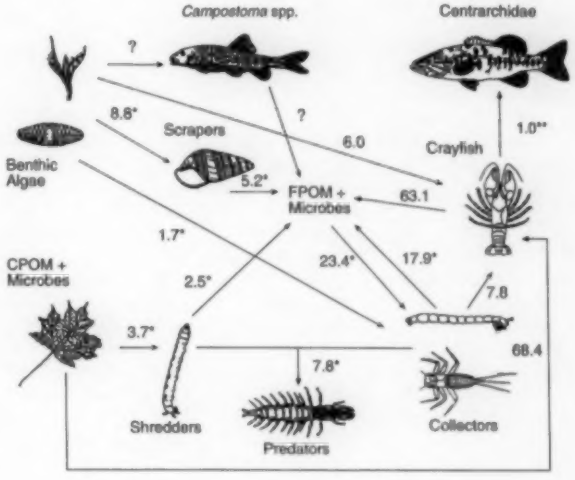
on benthic macroinvertebrates in this system, consuming as much animal matter annually as that estimated to be consumed by all other predatory benthic invertebrates combined (Rabeni et al. 1994). They also play an important role as shredders and consume about 18 times the amount of CPOM estimated to be processed annually by all other shredders combined (Rabeni et al. 1994). By increasing the surface area to volume ratio of CPOM, rendering it more susceptible to microbial attack and further breakdown, crayfishes may make CPOM more available as FPOM to the collector–filterer and collector–gatherer members of the benthic community (Cummins 1974). Since chironomids are typically collector–gatherers (Merritt and Cummins 1984), crayfishes in their role as shredders may be facilitating production of one of their most important prey items. Crayfishes also play a role as algal grazers, consuming roughly 50–60% of the amount of benthic algae estimated to be consumed annually by all other benthic invertebrates (Rabeni et al. 1994), although this does not account for the potentially large rate of algal consumption by *Campostoma* spp.

The accuracy of our production-based estimates of crayfish food consumption and egestion rates depends on the accuracy of AE calculated for crayfish feeding on each food type and the assumed value of NPE (0.5). Gravimetric techniques for determining AE do not account for weight losses of foods or fecal pellets due to leaching or microbial activity (Huryn and Wallace 1987), which may have biased our estimates of AE. Assimilation efficiencies of food types for *O. luteus* were similar to AE for other species of crayfish feeding on animal matter (Jones and Momot 1983) and plant material (Wiernicki 1984; Brown et al. 1986; Hessen and Skurdal 1988), although AE of detritus for *O. luteus* (14%) was somewhat lower than the maximum possible AE of detritus (33%) for *Cambarus bartonii* (Huryn and Wallace 1987). Assimilation efficiencies of animal matter, algae, and detritus for *O. luteus* were similar to AE for aquatic insects feeding on these food types (Lawton 1970; Anderson and Grafius 1975; Cummins 1975; Berrie 1976; Heiman and Knight 1976; McCullough et al. 1979; Benke and Wallace 1980). The value of NPE used in this study is consistent with NPE for aquatic insects (Edington and Hildrew 1973; McCullough 1975) and the crayfish *Cherax destructor* (Musgrove 1992) but is somewhat higher than NPE for other crayfish species (Mason 1974; Jones and Momot 1983). While estimates of the total amount of each food type consumed annually by crayfish would change if NPE was not 0.5, the relative proportions of food types consumed would remain the same as long as NPE is equivalent for all food types. Food consumption estimates of this type also assume equal rates of digestion for each food type (Benke and Wallace 1980). Foods that are digested more rapidly than others would be underrepresented in gut contents and in their contribution to crayfish production. Information on differential digestion rates of various food types would permit refinement of estimates of crayfish food consumption and egestion rates from this study.

In summary, crayfishes in the Jacks Fork River play multiple functional roles but are probably most important as shredders and predators, because they obtain most of their energy from feeding on invertebrates and terrestrial plant detritus and consume as much or more of these materials as the rest of the benthic invertebrate community combined. Crayfishes also constitute the majority of invertebrate biomass in this system and are the most energetically important component in the diets of smallmouth bass (*Micropterus dolomieu*) and rock bass (*Ambloplites rupestris*), both of which are valuable as sport fish (Rabeni 1992). By strongly influencing populations at several trophic levels, crayfishes have the potential to dominate energy flow in this system and act as "keystone" species. Further evaluation of the impacts of crayfishes on biomass and production of stream benthos is clearly needed.

Fig. 3. Conceptual model of major energy flow pathways in the Jacks Fork River involving crayfishes and comparison of organic matter consumption and egestion by crayfishes in relation to the rest of the benthic community. All values are in $g \cdot m^{-2} \cdot year^{-1}$. Values with a single asterisk are from Rabeni et al. (1994) and those with a two asterisks are from Rabeni (1992). The rest are from this study.



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Modifications of the reproductive period in mummichog (*Fundulus heteroclitus*) living downstream from a bleached kraft pulp mill in the Miramichi Estuary, New Brunswick, Canada

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Abstract: We evaluated if time of onset, intensity, and duration of the spawning period were modified in mummichog (*Fundulus heteroclitus*) exposed to bleached kraft pulp mill effluents (BKME). Gonadosomatic index (GSI), percentage of mature fish, fecundity, and egg size were measured in mummichog sampled weekly between May and August 1994 at 4 (M1), 21 (M2), and 39 km (M3) downstream from a bleached kraft mill in the Miramichi Estuary and at two sites located upstream (B1) and 20 km downstream (B2) in a reference estuary, the Bouctouche Estuary. In the Miramichi Estuary, the onset of gonadal maturation was delayed and egg size was reduced at M1 and M2 compared with M3 whereas these parameters did not vary between sites in the Bouctouche Estuary. Fish sampled at M1 demonstrated a marked increase in reproductive investment, with higher fecundity and GSI at reproduction maxima, compared with the other sites. This response went against the upstream–downstream trend observed at other sites in both estuaries. Condition factor was not reduced at M1, suggesting that more nutrients were available than at other sites. Sources of contamination other than BKME and natural factors may contribute to these responses.

Résumé : Nous avons évalué si le début, l'intensité et la durée de la période de ponte étaient modifiés chez des choquemorts (*Fundulus heteroclitus*) exposés aux effluents d'une usine de pâtes et papiers kraft à blanchiment au chlore (KBC). Le rapport gonadosomatique (RGS), le pourcentage de poissons matures, la fécondité et la taille des oeufs ont été mesurés chez des choquemorts capturés hebdomadairement, entre mai et août 1994, à 4 (M1), 21 (M2) et 39 km (M3) en aval de l'usine de pâtes et papiers kraft à blanchiment au chlore dans l'estuaire de la Miramichi et à deux sites, en amont (B1) et distant de 20 km en aval (B2), dans un estuaire de référence, l'estuaire de la Bouctouche. Dans l'estuaire de la Miramichi, le début de la période de reproduction était retardé et la taille des oeufs inférieure à M1 et à M2, comparé à M3, alors qu'il n'y avait pas de différence dans l'estuaire de la Bouctouche. L'investissement reproducteur des poissons capturés à M1 était plus intense qu'ailleurs, alors que la fécondité et le RGS étaient supérieurs aux maxima de reproduction, et allait à l'encontre de la tendance amont–aval observée dans les deux estuaires. Le facteur de condition n'était pas inférieur à M1, suggérant que la nourriture était plus disponible qu'ailleurs. Des sources de contamination autres que les effluents KBC et (ou) des facteurs naturels peuvent aussi expliquer les réponses observées.

Introduction

Bleached kraft pulp mill effluents (BKME) discharged into the environment at sublethal concentrations may affect fish at different levels (Owens 1991). Among the possible effects, changes in reproduction are prime concerns and a major endpoint in environmental monitoring. Such changes could have a dramatic impact on the persistence of populations (Moriarty 1985; Ramade 1992).

Many field studies have documented alterations in the reproduction of fish exposed to BKME, including lower recruitment (Neuman and Karas 1988; Karas et al. 1991), delayed gonadal maturation (Andersson et al. 1988*a*; Larsson et al. 1988), smaller gonads (Sandström et al. 1988; Munkittrick et al. 1991, 1994), and masculinization of females (Howell et al. 1980; Denton et al. 1985). Laboratory studies also showed lower egg production and delayed reproduction in fathead minnow (*Pimephales promelas*) exposed to BKME (NCASI 1985; Kovacs et al. 1995). Furthermore, disruptions of the pituitary–gonadal axis at many sites were demonstrated in white sucker (*Catostomus commersoni*) exposed to BKME (Van der Kraak et al. 1992), leading to a reduction of plasma concentrations (Munkittrick et al. 1991; Van der Kraak et al. 1992; Gagnon et al. 1995) and a decreased capacity of steroid biosynthesis by the ovaries and an altered peripheral metabolism of steroids (Van der Kraak et al. 1992).

The discharge of BKME into the environment is controlled by companies and governments. Canadian regulations, set out

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Table 1. Comparison of the Miramichi and Bouctouche estuaries.

Parameter	Miramichi Estuary	Bouctouche Estuary
Population (habitants)	50 000	10 000
Drainage basin area (km^2)	14 000	426
Length (km)	80	25
Width at the mouth (km)	20	1
Size (km^2)	300	33
Mean annual discharge ($m^3 \cdot s^{-1}$)	313	na
Location of sampling sites	M1: 46°59′N, 65°34′W	B1: 46°24′N, 64°50′W
	M2: 47°04′N, 65°20′W	B2: 46°29′N, 64°40′W
	M3: 47°02′N, 65°10′W	

Note: na, not available. Information on the Miramichi Estuary was taken from Courtenay et al. (1995) and from New Brunswick Department of the Environment (1991) and information on the Bouctouche Estuary was taken from R. Boudreau (New Brunswick Department of Fisheries and Aquaculture, Bouctouche, N.B., personal communication).

under the *Fisheries Act*, stipulate that pulp and paper mills must undertake an "Environmental Effects Monitoring" program (Environment Canada 1992). An adult fish survey is required to provide information on morphological and life history characteristics of two sentinel species of fish living near the outlet and in an unexposed reference site. Growth, reproductive output, age structure, and condition are assessed in adult fish sampled once, at the time of gonadal maturation. While considerable information exists on the selection of sentinel species and on the design of such surveys in freshwater environments, information is lacking for estuarine and marine environments. In estuaries, the bidirectional movement of water caused by the tides adds to the complexity of the environment, when compared with the unidirectional flow found in the freshwater system. Moreover, many species of fish are unsuitable for site-specific monitoring studies because of their extensive movements and an incomplete knowledge of their life histories.

Laboratory studies and European field studies have demonstrated that BKME may alter different stages of the reproductive cycle (NCASI 1985; Sandström et al. 1988; Karas et al. 1991; Kovacs et al. 1995). The present study proposes a method to evaluate multiple stages of the reproductive cycle in estuarine fish exposed to BKME by sampling fish intensively. Mummichog (*Fundulus heteroclitus macrolepidotus*) is used as a sentinel species because it is sedentary, abundant, easy to capture, with a well-documented reproductive physiology (Fritz et al. 1975; Lotrich 1975; Taylor et al. 1979; Eisler 1986; Leblanc and Couillard 1995) and because sequential sampling during the whole reproductive period at multiple sites is possible with such a species. An epidemiological approach, with multiple reference sites, is used to control for the effect of natural factors on fish reproduction.

This study was conducted to test the null hypothesis that mummichog living immediately downstream from a bleached kraft mill showed no differences in time of onset, intensity, and duration of the reproductive period, compared with fish from a reference estuary.

Materials and methods

Study design

Time of onset, intensity, and duration of the reproductive period, gonadosomatic index (GSI), fecundity, egg size, percentage of ripe fish, and condition were measured to compare reproductive investment in mummichog among sites within the Miramichi and Bouctouche estuaries. A previous study (Couillard and Légaré 1994) had shown that mummichog captured downstream from a bleached kraft mill in the Miramichi Estuary exhibited symptoms commonly associated with BKME exposure (Owens 1991). Increased prevalence of fin erosion and vertebral malformations and a 2.5-fold induction of the activity of the hepatic ethoxyresorufin *O*-deethylase (EROD) were observed at M1 compared with M3 (see next section for locations), suggesting differential exposure to contaminants (Couillard and Légaré 1994). In the Bouctouche Estuary, EROD activity did not differ among sites (Linda McCarthy, Canada Centre for Inland Waters, Burlington, ON L7R 4A6, Canada, personal communication). In this study area, it was not possible to find a reference estuary of the size of the Miramichi Estuary. Since the Bouctouche Estuary is almost 10 times smaller than the Miramichi Estuary (Table 1), the assumption was made that the difference in size between the estuaries would cause a smaller effect on the upstream–downstream variations of reproductive parameters than the effect caused by the contamination in the Miramichi Estuary. Thus, responses possibly associated with contamination would stand out, by their nature or intensity, from the natural variations in reproduction caused by ecological gradients.

Numerous natural factors including water temperature, food supply, photoperiod, age, and size of fish may influence fish reproduction (Lam 1983; Kneib 1986; Jobling 1994). In the Miramichi Estuary, there is an upstream–downstream ecological gradient parallel to the BKME contamination gradient. To control for the effects of natural factors on fish reproduction, (1) spatial variations of reproductive parameters along upstream–downstream gradients were compared between the contaminated Miramichi Estuary and the reference Bouctouche Estuary, (2) sampling of mummichog was stratified by length to control for the effect of size on reproduction, and (3) water temperature, salinity, and condition factor (CF) (as an indicator of food availability) were measured at each site throughout the sampling period and compared among sites.

Study areas

The Miramichi Estuary (New Brunswick, Canada; Fig. 1) is contaminated by the effluents of a secondary-treated bleached kraft pulp mill located in Newcastle (Repap NB, Inc., Kraft Pulp and Paper Mill). High levels of chlorinated compounds were discharged into the estuary from the mill (New Brunswick Department of the Environment 1991) before the introduction of chlorine dioxide as a partial substitute for molecular chlorine in May 1993. These two chemicals are now used as bleaching agents in the first stage of the bleaching process and chlorine dioxide alone in the two latter stages. The bleaching sequence is $(C50/D50)E_{o,p}DE_p$ where C50/D50 is 50% molecular chlorine (Cl_2) and 50% chlorine dioxide, $E_{o,p}$ is a caustic extraction with oxygen and peroxide, D is chlorine dioxide, and E_p is a caustic extraction with peroxide. In 1994, based on the annual production, the

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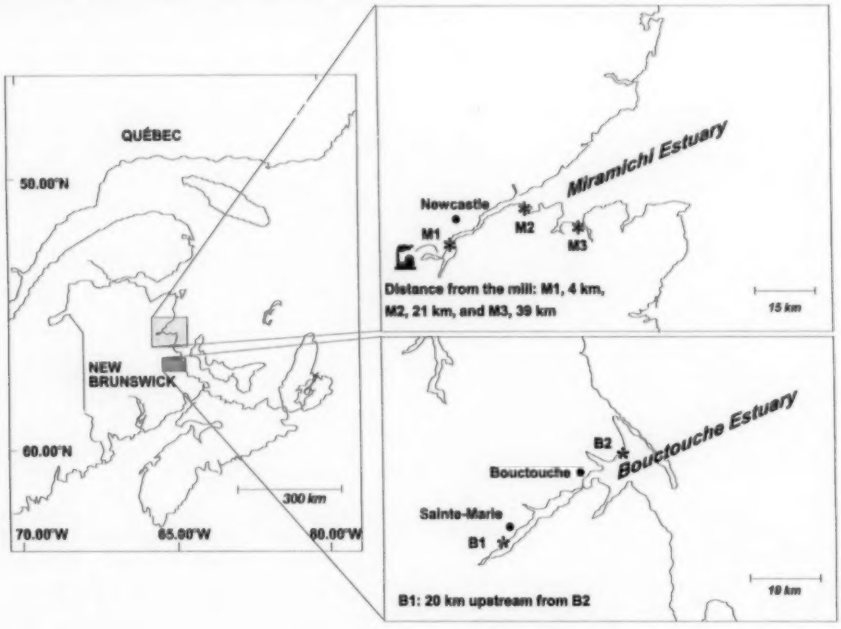
Fig. 1. Sampling sites in the Miramichi Estuary (M1, M2 and M3) and in the Bouctouche Estuary (B1 and B2). In the Miramichi Estuary, the symbol upstream from M1 represents the bleached kraft pulp mill.



daily production was 1306 air-dry metric ton of paper and the mean flow discharged into the estuary was 72 923 $m^3 \cdot day^{-1}$ (O. Kristiansen, Repap N.B. Inc., Newcastle, NB E1V 3N3, Canada, personal communication). In addition to the kraft mill, other sources of contamination in the Miramichi Estuary include a primary-treated groundwood mill, municipal effluents from different localities, mining activities (zinc and copper) upstream in the Northwest Miramichi River, and a former Domtar wood-preserving plant in Newcastle (New Brunswick Department of the Environment 1991). However, BKME is the principal source of organic contaminants into the estuary (Courtenay et al. 1995). Sampling sites in the Miramichi Estuary are located downstream from the kraft mill at 4, 21, and 39 km for M1, M2, and M3, respectively (Fig. 1). The sources of contamination are located both upstream and downstream from M1, but are all upstream from M2. M1 is located near the upstream limit of distribution of mummichog and M3 is at the mouth of the estuary. All sites are intertidal marshes and have a sandy bottom, except M1, where the bottom is muddy and rocky.

The Bouctouche Estuary is located 100 km south of the Miramichi Estuary and is separated from the Strait of Northumberland by a long dune (Fig. 1). Contamination in the Bouctouche Estuary comes principally from agricultural activities at points along the length of the estuary and river. Municipal effluents from Bouctouche and Sainte-Marie are also discharged into the Bouctouche system (R. Boudreau, New Brunswick Department of Fisheries and Aquaculture, Bouctouche, NB E0A 1G0, Canada, personal communication). B1 is located at Sainte-Marie, near the upstream limit of distribution of mummichog, in an intertidal marsh with sandy bottom. At this site, the estuary is approximately 200 m wide. B2 is located 20 km downstream, near the mouth of the estuary. The bottom of this site is composed of sand covered with brown algae.

Sampling procedures

At each site, fish were captured weekly from May to August 1994 using four to six minnow traps baited with capelin. The traps were deployed for a period of approximately 20 h and removed the day after. Water temperature and salinity were measured at the time of sampling with a multiprobe sensor (Grant/YSI 3800 Water Quality Logging System, YSI Inc.). Each fish captured was measured (total length to the nearest millimetre) and sorted by sex and length-class. A minimum length of 6.5 cm was set to ensure that all fish examined were adults. Mummichog reach maturity when total length is approximately 4 cm (U.S. Fish and Wildlife Service 1985). Fifteen males and 15 females were randomly selected within three length-classes (6.5–7.4, 7.5–8.4, and 8.5–9.4 cm total length).

The selected fish were brought back to the laboratory alive and anaesthetized with 175 mg MS 222·L^{-1} (tricaine methanesulfonate, Sigma Chemical Co., St. Louis, Mo.). Total length, total mass, gonad mass, and carcass mass (total mass less gut, internal organs, and gonads) were measured. The gonadal stages of maturity of females were established from morphological criteria (Selman and Wallace 1986), and gonads of ripe females (stage 5) were preserved in 4% phosphate-buffered formaldehyde for subsequent measurement of fecundity and egg size. Ripe males could not be adequately discriminated from unripe males by morphological criteria. Therefore, those with a GSI greater than 2.0 were designated as ripe (Kneib and Stiven 1978; Taylor 1986; Leblanc and Couillard 1995).

During the reproductive period, males develop a yellow coloration on their abdomen as a secondary sexual characteristic (Newman 1907; U.S. Fish and Wildlife Service 1985). A coloration index was used to estimate the intensity of the coloration, graded using a color chart as 1 corresponding to pale yellow, 2 and 3 to intermediate yellows, and 4 to dark yellow.

Reproductive parameters

Percentages of ripe fish were calculated for each week. GSI and CF were calculated for each fish as follows: GSI = 100 × [gonad mass (g)/(total mass (g) – gonad mass (g))] and CF = 100 × [carcass mass (g)/(total length (cm))^3].

In multiple spawners such as mummichog, percentages of ripe fish and their fecundity fluctuate during the reproductive period with peaks immediately before spawning events (Taylor et al. 1979; Kneib

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1986). To follow spawning activity of the population sampled during the whole season, a reproductive index (RI) was determined for each sex, site, and week of sampling according to the following formula: RI = (median GSI of ripe fish × percentage of ripe fish). At each sampling site, maximal RI was used as an indicator of maximal egg production in the sampled population. During the whole reproductive period, the area under the graph of RI as a function of time was measured at each site using an image analyzer system (BIOQUANT, R&M Biometrics®). The area represents an integrated view of the total investment in development of the sexual organs during the whole reproductive period, since it takes into account the variations in percentages of ripe fish as well as the relative size of their gonads. Reproductive investment would be higher at sites where high percentages of ripe fish are observed more frequently and where fecundity, indicated by GSI of ripe fish, is high. We assumed that mummichog did not maintain mature ova beyond the expected time of spawning. Such an effect has never been observed in fish exposed to pulp and paper effluent (Owens 1991; Kovacs et al. 1995). We did conduct fertilization experiments with females collected from all sites. Fertilization rates were high and did not differ significantly among sites (C.M. Couillard, unpublished data). Abnormal egg retention would have caused a deterioration of egg quality and reduced fertilization rates.

To evaluate if there was delayed gonadal maturation at the site closest to the mill, GSI, percentage of ripe fish, and nuptial coloration were calculated at each site at the beginning of the reproductive season (weeks 2–4 combined, May 24 to June 10, 1994). The first week of sampling was not considered because an insufficient number of individuals were collected at some sites.

To evaluate if the maximal reproductive potential of fish differed among sites, GSI and percentage of ripe fish were calculated at the weeks when RI was the highest (reproduction maxima) for each sex and site. For each parameter tested, only the weeks that did not differ statistically and corresponded to higher levels of reproductive parameters were combined for the among-site comparisons. Fecundity was measured in all ripe females examined at reproduction maxima. Mature eggs were big and translucent with a pale yellow coloration, lipid droplets at one pole, and chorionic filaments (Kneib 1986). These eggs were counted with a binocular microscope to determine the potential fecundity of a single female. Opaque eggs were not counted, since their stage of maturity and their viability could not be determined adequately. When opaque eggs were present, they typically accounted for less than 5% of the total number of eggs per female. A subsample of 20 mature eggs was selected randomly and diameters were measured with an image analyzer system.

Data analyses

The objective of having an equal number of fish per length-class at each site was not attained throughout the 12 weeks of the sampling period (data not shown; for more details, see Leblanc 1997). Thus, to evaluate if size influenced the parameters being analyzed, fecundity, percentage of ripe fish, the relationship between gonad mass and somatic mass (total mass minus gonad mass), and the relationship between carcass mass and total length were compared among length-classes for each sex and site. In the Bouctouche Estuary, males and females from the length-class 6.5–7.4 cm had a different relationship between carcass mass and total length compared with males and females from other length-classes. This class was withdrawn only for comparisons of CFs among sites within the Bouctouche Estuary. For other parameters, no differences were present among length-classes and all length-classes were combined to carry out the among-week and among-site comparisons.

Temporal variations of percentage of ripe fish, CF, and reproductive investment were described and compared among sites on a qualitative basis. Temperature and salinity, at the beginning of the reproductive period, and fecundity and egg size, at reproduction maxima, were compared among sites, within each estuary, using the Kruskal–Wallis test. When significant differences were detected, this was followed by a one-way analysis of variance on a ranked data set with Tukey's test (Conover 1980). At the beginning of the reproductive period and at reproduction maxima, Fisher's exact test was used to compare the percentages of ripe fish among sites, within the same estuary, and the Mann–Whitney *U*-test was used to compare the coloration indices among sites. Comparisons of the relationships between carcass mass and total length were performed using log-transformed data. Slopes and intercepts of these relationships and of the relationships between gonad mass and somatic mass were compared among sites using an analysis of covariance.

Differences were considered significant when $p \leq 0.05$, except for the length-class comparisons ($p \leq 0.01$), as a large number of comparisons were made. All statistical analyses were performed using the SAS statistical computing system, release 6.08 (SAS Institute Inc. 1989).

Results

Whole and beginning of the reproductive season

In the Miramichi Estuary, a high percentage of ripe fish (more than 50%) was observed less frequently and for a shorter period of time at M2 compared with the downstream site (M3) and with the site closest to the mill (M1) (Fig. 2). In the Bouctouche Estuary, a high percentage was observed less frequently and for a shorter period of time at the upstream site (B1) compared with the downstream site (B2), although no fish were captured at the latter site after the ninth week of sampling (July 13, 1994). After that date, B2 was subjected to large deposits of algae brought in with each incoming tide.

During the whole reproductive season, reproductive investment of males and females was higher at M1. This was indicated by an area under the graph of RI as a function of time that was 2 to 3 times higher in females from M1 compared with M2 and M3 (Fig. 3). Reproductive investment was 1.4 times higher in females from M3 and B2 (downstream sites) compared with females from the upstream sites M2 and B1. Breeding effort of males from M1 was approximately 1.6 times that of fish from the other sites in the Miramichi Estuary (Fig. 3).

The reproductive season began later at M1 and M2, 1–2 weeks after M3, B1, and B2. During the first weeks of the reproductive season, the percentage of ripe females was 1.5 and 2 times higher at M3 than at M2 and M1, respectively ($p \leq 0.05$; Table 2). Fifty percent of the females were ripe at M3 during the third week of sampling whereas this occurred at M2 and M1 during the fourth and fifth week, respectively. Males showed a less intense nuptial coloration at M1 and M2 than at the other sites ($p < 0.05$; Table 3).

In the Miramichi Estuary, females from M3 exhibited a smaller carcass mass for a similar length compared with females from other sites (intercepts, $p = 0.013$; Table 4) whereas males showed no differences among sites (intercepts, $p = 0.13$). In the Bouctouche Estuary, males and females from B2 had smaller carcass mass for a similar length than at B1 (intercepts, $p = 0.008$ and $p = 0.037$, respectively; Table 4). For each sex, within each estuary, there were little differences among sites in CF over the whole reproductive season (Fig. 4). However, CF of fish from downstream sites (M3 and B2) tended to be lower than of those from the other sites.

At the beginning of the reproductive season, temperature did not vary among sites in both estuaries, but salinity was lowest at M1 ($p = 0.006$; Table 5). During the first 7 weeks of

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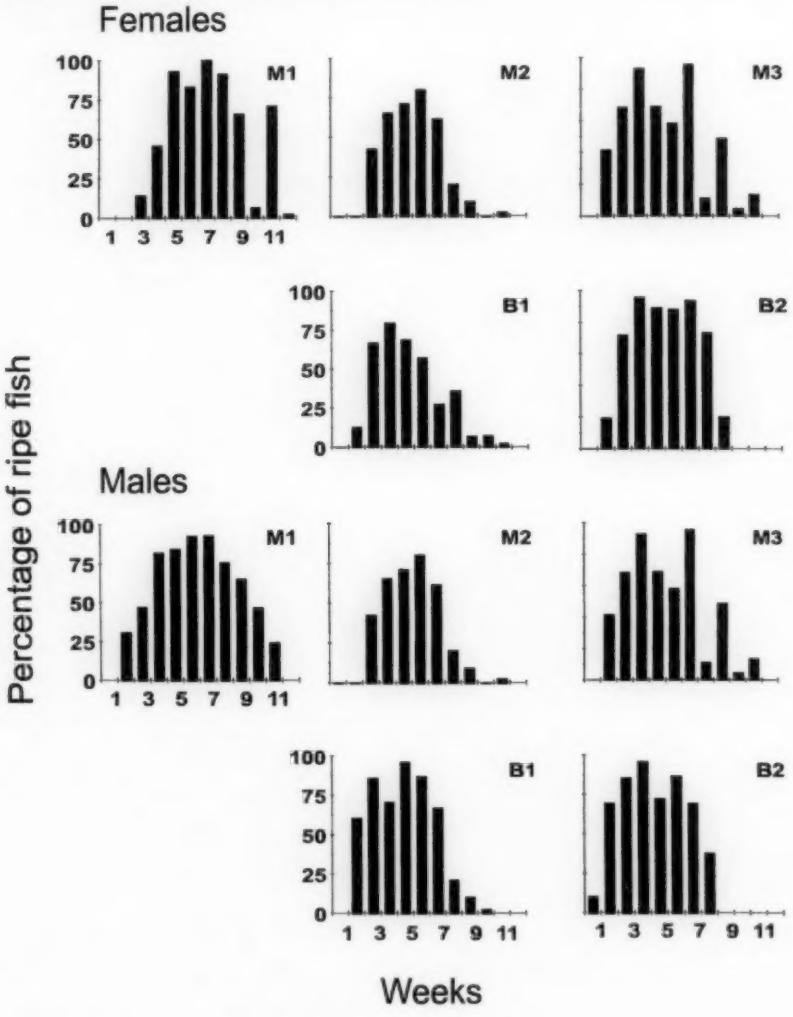
Fig. 2. Temporal variations of the percentage of ripe female and male mummichog at each site in the Miramichi and Bouctouche estuaries, from May 17th (week 1) to July 25th (week 11). See Table 2 for statistical analyses.



sampling, temperature increased gradually at each site and the temperature ranges were very similar among sites (Table 5). A salinity gradient was present in both estuaries (Table 5). There were larger variations in salinity at downstream sites (M3 and B2), and the salinity maximum was markedly lower at M1 in the Miramichi Estuary. No data are available after the seventh week.

Reproduction maxima

In the Miramichi Estuary, the rate of increase of ovarian mass relative to somatic mass was two times higher in females from M1 compared with other sites (slopes, $p = 0.001$; Table 6). In the Bouctouche Estuary, females from B1 had bigger ovaries for a similar somatic mass than those from B2 (intercepts, $p = 0.02$; Table 6).

At reproduction maxima, males from M1 had bigger testes for a similar somatic mass than those from M3 (intercepts, $p = 0.02$; Table 6). Males from B1 had smaller testes for a similar somatic mass than those from B2 (intercepts, $p = 0.008$; Table 6).

In the Miramichi Estuary, fecundity was highest at M1, where females displayed twice the fecundity reached by females at M2 ($p \leq 0.001$; Table 7). Fecundity was 1.4 times higher at M3 than at M2. Egg diameter increased from upstream to downstream sites in the Miramichi Estuary ($p \leq 0.001$; Table 7), but not in the reference estuary ($p = 0.08$).

The percentage of ripe females was lower at M2 ($p \leq 0.01$; Table 2) whereas there was no difference between M1 and M3 ($p = 0.32$). In the reference estuary, the percentage of ripe females was lower at B1 than at B2 ($p \leq 0.001$).

The intensity of nuptial coloration of males was higher at M1 than at M3 ($p = 0.007$; Table 3).

Discussion

This study has shown that (1) mummichog from M1 and

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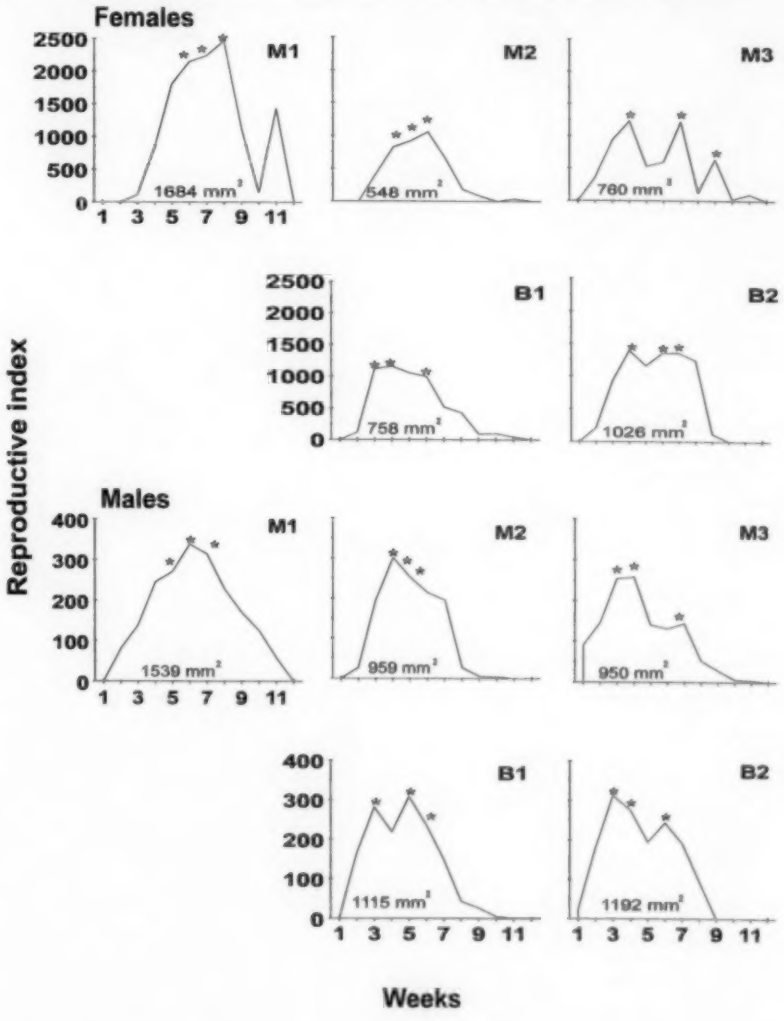
Fig. 3. Temporal variations of the reproductive index (RI) of ripe female and male mummichog in the Miramichi and Bouctouche estuaries, from May 17th (week 1) to July 25th (week 11). The numbers represent the area under the curve indicating breeding effort of ripe fish (the area was measured against different scales for males and females). Asterisks correspond to reproduction maxima.



M2 exhibited a delayed onset of the reproductive period and had smaller eggs, compared with M3, whereas no differences were observed between sites in the Bouctouche Estuary, (2) the investment in reproduction by mummichog during the reproductive period was more intense at M1 (demonstrated by higher GSI, fecundity, and breeding effort) and this response went against the upstream–downstream trend observed in both estuaries, and (3) the spawning period was not shorter at M1, but was shorter at M2 and B1 than at M3 and B2, in the Miramichi and Bouctouche estuaries, respectively.

In the Miramichi Estuary, gonadal maturation was delayed at the two most upstream sites (M1 and M2) whereas this upstream–downstream trend was not observed in the reference estuary. Anthropogenic or natural factors could be responsible for the delay seen at M1 and M2. Delayed gonadal maturation has been observed previously in fish exposed to BKME in field studies (Andersson et al. 1988*b*; Sandström et al. 1988; Munkittrick et al. 1991, 1994; Gagnon et al. 1995) and in laboratory studies (Kovacs et al. 1995). These responses are not specific to BKME, however, and could also be related to other contaminants (Kime 1995).

Among natural factors, temperature is one of the most important factors influencing the maturation of the gonads of fish (Lam 1983; Day and Taylor 1984; Taylor 1986). At the beginning of the reproductive season, temperature did not differ among sites of capture. These shallow sampling locations are probably influenced most by air temperature. Thus, water temperature rose continuously during the study period and did not differ among sites. Fish may be exposed to different conditions when they move out of the intertidal marshes into the main channels. Wallace and Selman (1981) found that most female mummichog were carrying eggs when offshore temperature rose above 11°C, near Woods Hole, Mass. Lafleur et al. (1995) monitored temperature in the Miramichi Estuary in 1992 and

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Table 2. Percentage of ripe mummichog (sample size in parentheses) at the beginning of the reproductive season and at reproduction maxima at different sites in the Miramichi and Bouctouche estuaries.

	Estuary	Site	Beginning	Maxima
Females	Miramichi	M1	28 (13) A	87 (121) A
		M2	37 (39) A	70 (66) B
		M3	56 (78) B	91 (105) A
	Bouctouche	B1	51 (66)	69 (82) a
		B2	52 (69)	92 (109) b
Males	Miramichi	M1	64 (48)	88 (74)
		M2	52 (52)	89 (79)
		M3	63 (105)	83 (72)
	Bouctouche	B1	67 (84)	89 (73)
		B2	71 (119)	89 (122)

Note: Different letters indicate significant differences among sites within each estuary (Fisher's exact test, $p \leq 0.05$).

Table 3. Percentage (sample size in parentheses) of male mummichog with the most intense nuptial coloration (indices 3 and 4) at the beginning of the reproductive period and at reproduction maxima at each site in the Miramichi and Bouctouche estuaries.

Site	Beginning	Maxima
M1	26 (18) A	85 (70) A
M2	36 (30) A	76 (64) AB
M3	44 (53) B	73 (77) B
B1	45 (50)	61 (67)
B2	45 (59)	71 (95)

Note: Different letters indicate significant differences among sites in the Miramichi Estuary (Mann–Whitney *U*-test, $p \leq 0.05$).

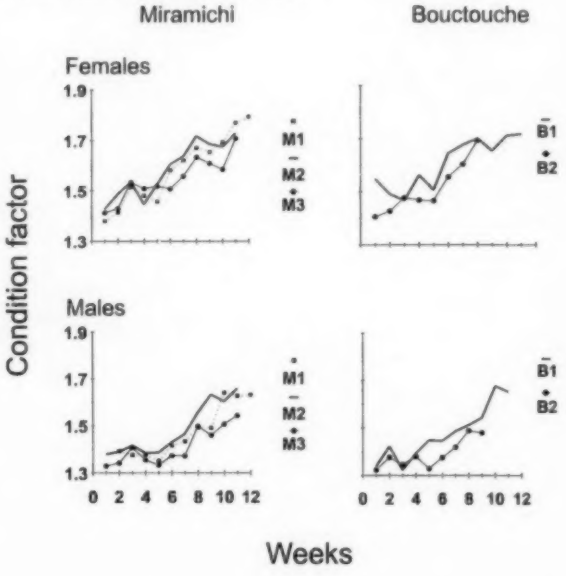
Table 4. Analyses of covariance of carcass mass of mummichog as a function of total length and site in the Miramichi and Bouctouche estuaries at the beginning of the reproductive period.

	Miramichi			Bouctouche	
	M1	M2	M3	B1	B2
Females					
N	42	91	102	71	93
Slope	3.28	3.28	3.28	3.37	3.37
Intercept	–2.29A	–2.29A	–2.30B	–2.38a	–2.39b
F (*p*)		8.91 (0.0002)		4.41 (0.0373)	
Males					
N	67	98	114	80	106
Slope	3.25	3.25	3.25	3.32	3.32
Intercept	–2.24	–2.24	–2.25	–2.29a	–2.30b
F (*p*)		1.18 (0.31)		7.27 (0.0077)	

Note: Carcass mass and total length are log-transformed data. *N* indicates the number of fish. Homogenized slopes are shown here. For each sex, different letters indicate significant differences among sites within each estuary.

found that the offshore water temperature reached 11°C at upstream sites at least 2 weeks before stations located further downstream. The delay in gonadal maturation observed in this study had already been observed at M1 in 1992 (C.M. Couillard, personal observation). Thus, low water temperature is probably not the cause of the delay in gonadal maturation observed at upstream sites in the Miramichi Estuary. The availability and quality of food could also affect the gonadal maturation of fish at the beginning of the reproductive season, but the condition of fish was not lower at the sites where a delay in gonadal maturation was observed. Thus, although natural factors cannot be totally excluded, our observations suggest an anthropogenic cause for the delayed gonadal maturation.

Fig. 4. Temporal variations of the condition factor (CF) of female and male mummichog at each site in the Miramichi and Bouctouche estuaries, from May 17th (week 1) to July 25th (week 11). See Table 4 for statistical analyses.



Despite the delay in the onset of reproduction, fish living near the bleached kraft mill invested more intensely in reproduction than fish from the other sites, as reproductive investment, gonad mass, and fecundity were considerably higher at M1. At this site, the reproductive investment of mummichog was also higher during the whole spawning season. Despite the high investment in reproduction, the CF of fish from M1 was very similar to that of fish from the other sites and increased continuously throughout the reproductive period. Thus, the most likely explanation for the increased investment in reproduction at M1 is higher food availability with greater energetic resources put into reproduction without having to sacrifice condition (Jobling 1994). At M1, eutrophication may be responsible for the associated nutrient enrichment and could have multiple sources: BKME (New Brunswick Department of the Environment 1991; Owens 1991; Bothwell 1992), municipal effluents, and (or) groundwood mill effluents (New Brunswick Department of the Environment 1991). It is also possible that habitat deterioration or difficult environmental conditions could have reduced the density of fish at this site. A reduced fish density has previously been observed in the vicinity of a bleached kraft mill (Neuman and Karas 1988). This situation may have enabled mummichog to feed in a habitat where nutrients were more available as a result of reduced competition for food. Alternatively, the same conditions could have selected, over generations and as a mechanism of

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Table 5. Median temperature and salinity (minimum–maximum and number of observations in parentheses) at the beginning of the reproductive season and range of median temperature and median salinity during the sampling period at each site in the Miramichi and Bouctouche estuaries.

	Beginning		Range	
Site	Temperature (°C)	Salinity	Temperature (°C)	Salinity
M1	16.5 (13.0–22.3; 7)	0.3 (0.2–0.7; 7)A	8.3–19.6	0.2–1.4
M2	18.0 (12.0–21.1; 12)	1.9 (1.5–7.4; 12)B	11.1–23.8	0.8–9.6
M3	14.9 (11.2–19.1; 13)	2.3 (0.4–5.7; 13)B	9.7–16.2	0.2–20.9
B1	13.0 (11.1–21.6; 14)	1.8 (0.5–6.4; 14)	10.7–21.3	0.1–7.9
B2	13.7 (8.2–20.8; 16)	3.0 (0.3–14; 16)	13.1–21.9	1.9–21.4

Note: The range corresponds to the temperature measured at weeks 1 and 7, and the range of salinity corresponds to the minimal and maximal salinities measured between those two weeks. Different letters indicate significant differences among sites (Kruskal–Wallis test, $p \leq 0.006$).

Table 6. Analyses of covariance of gonad mass of mummichog as a function of somatic mass and site in the Miramichi and Bouctouche estuaries at reproduction maxima.

	Miramichi			Bouctouche	
	M1	M2	M3	B1	B2
	Females				
N	38	61	73	74	42
Slope	0.32 A	0.12 B	0.12 B	0.17	0.17
Intercept	–0.22	0.06	0.05	0.01 a	–0.13 b
F (*p*)		0.03 (0.86)		5.60 (0.0195)	
	Males				
N	73	38	61	42	74
Slope	0.03	0.03	0.03	0.03	0.03
Intercept	0.05 A	0.04 AB	0.03 B	0.01 a	0.09 b
F (*p*)		3.40 (0.0359)		28 (0.0001)	

Note: *N* indicates the number of fish. The slope relating gonad mass of females from M1 to somatic mass was different from those of females from M2 and M3. Intercepts and slopes were similar at M2 and M3. Except for females from M1, slopes were parallel among sites for each sex within each estuary. Homogenized slopes are shown here. For each sex, different letters indicate different intercepts among sites within each estuary.

Table 7. Fecundity and egg size (mean ± SD (*N* in parentheses)) of ripe female mummichog sampled at different sites in the Miramichi and Bouctouche estuaries at reproduction maxima.

Site	Fecundity	Egg diameter (mm)
M1	120±109 (120) A	1.61±0.06 (119) A
M2	66±39 (88) B	1.73±0.07 (78) B
M3	94±59 (94) C	1.81±0.06 (85) C
B1	79±52 (88)	1.71±0.08 (78)
B2	94±60 (150)	1.74±0.07 (116)

Note: Fecundity is the number of mature eggs. Different letters indicate significant differences among sites within the Miramichi Estuary (Kruskal–Wallis test, $p \leq 0.05$).

Table 8. Upstream–downstream trends in the reproductive parameters of female mummichog.

	Miramichi Estuary		Bouctouche Estuary
Characteristic	M1–M3	M2–M3	B1–B2
Fecundity	+[a]	–	–
Egg size	–	–	0
Breeding effort	+[a]	–	–
% ripe females	0[a]	–	–
Ovarian growth	+[a]	0	+
CF	+	+	+

Note: 0, no change; +, increase; –, decrease at upstream site relative to downstream site. Ovarian growth corresponds to the results of the analysis of covariance between ovarian mass and somatic mass at reproduction maxima.

[a]M1 showed tendencies opposite to the upstream–downstream trend observed at the other sites in both estuaries.

compensation, fish with the highest fitness to survive and to reproduce in an altered environment (Moriarty 1985).

Despite the large difference in size between the two estuaries, the spatial variations of the intensity and duration of the spawning period of mummichog were very similar when M2 and B1 were compared with downstream sites (M3 and B2) within each estuary (Table 8). Females from M2 and B1 invested less in reproduction than fish from sites further downstream (M3 and B2), as shown by a shorter spawning period, reduced fecundity, reproductive investment, and percentage of ripe females at reproduction maxima. At M2 and B1, a low nutrient availability, a higher competition for food, or a higher pressure of predation could have hindered the reproduction of fish (Jobling 1994).

At the site immediately downstream from the bleached kraft mill in the Miramichi Estuary, egg size was smaller compared with other sites. Reduction in egg size may be caused by an elevated fecundity, since egg size is negatively correlated with fecundity (Kartas and Quignard 1984), by exposure to contaminants (Munkittrick and Dixon 1988; Sandström et al. 1988; McMaster et al. 1991, 1994), or by low food availability (Jobling 1994). The last factor can be rejected, as the CF was not lower at M1 where the eggs were smaller than at M2 and M3. Geographical variations in egg diameter have also been associated with salinity (Marteinsdottir and Able 1988). However, decreased egg size is generally related to high salinity as a result of osmotic pressure, as described in *F. heteroclitus* and other species of fish (Solemdal 1967; Lee 1981; Morgan and Rasin 1982; Marteinsdottir and Able 1988). Larger eggs have been observed in populations of *F. heteroclitus* that deposit their eggs in the intertidal zone, in a location where the eggs are likely to become stranded out of water for a long period of time (Marteinsdottir and Able 1988). It is possible that in the Miramichi Estuary, the tidal influence on eggs is stronger at the most downstream site, M3. Intraspecific

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differences in egg size have also been related to female size and to season, but these factors were controlled in this study.

Reproduction of mummichog appeared enhanced at the site immediately downstream from the bleached kraft mill. However, all aspects of fish reproduction were not evaluated in this study. It is possible that recruitment may be altered if hatching success, embryonic survival, or larval survival is reduced. Also, other species of fish may be more affected than mummichog, which is known to be a very plastic and adaptable species of fish (Eisler 1986). In the Great Lakes, Munkittrick et al. (1992) showed that white sucker and lake whitefish (*Coregonus clupeaformis*) exposed to BKME exhibited depressed steroid levels, delayed sexual maturity, and changes in reproduction whereas longnose sucker (*Catostomus catostomus*) exhibited only depressed steroid levels with no apparent impacts on reproductive performance.

In conclusion, despite a delayed gonadal maturation, fish from the site closest to the bleached kraft mill increased their investment in reproduction. Our results suggest anthropogenic influence on fish reproduction because fish from M1 exhibited responses that did not correspond to the upstream–downstream trend observed in both estuaries and because the observed responses have been previously associated with exposure to various contaminants including BKME. However, the cause of these changes cannot be conclusively assigned to BKME, as other sources of contamination and natural factors present cannot be entirely eliminated. Moreover, it is not possible with this study to distinguish past from current contamination. Evaluation of the reproduction of mummichog exposed to BKME in the laboratory is necessary to assess the direct effect of the effluent on the onset of maturation, fecundity, and egg size.

This study clearly showed the importance of sampling at more than one moment during the reproductive cycle to evaluate the impact of contaminants on the reproduction of estuarine fish. The reproduction of mummichog would have appeared altered if only one sample had been taken during gonadal maturation and would have appeared enhanced if sampling had occurred during the reproduction maxima. Mummichog, because it is abundant, sedentary, and small, proved to be a very useful species on which to conduct such a study. Further studies on age-at-maturity, quality and viability of eggs, development and survival rates of early life stages, fertilization rates, and impact of eutrophication on fish reproduction are needed for a complete field evaluation of the reproductive cycle. Additional field studies are also needed to determine if the responses observed in mummichog are encountered in other species in the Miramichi Estuary.

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N and P limitation of benthos abundance in the Nechako River, British Columbia

Chris J. Perrin and John S. Richardson

Abstract: Additions of inorganic N and P to replicated stream mesocosms were used to test the hypothesis that production of the benthic food web in the flow-controlled Nechako River, B.C., was N and P limited. N, P, and N + P additions were contrasted with controls to determine the responses of periphyton and macroinvertebrates over a 33-day period in late spring. Periphyton accrual under N + P addition was greater than two times that of the controls, and N alone produced a moderate increase. Periphyton showed relatively little response to P addition compared with controls. Macroinvertebrates, especially orthocladiine midges and mayflies, increased in density under N or N + P additions, in contrast with P additions and controls. Increased emergence rates of adult aquatic insects from the mesocosms were consistent with increases observed in benthic invertebrates. The results showed nutrient limitation of benthic productivity and that algal and benthic invertebrate responses were closely coupled. N was most limiting, but with additions of inorganic N as low as 10 $\mu g \cdot L^{-1}$, P became limiting. The benthic invertebrates that increased most in abundance corresponded to the predominant prey in chinook salmon (*Oncorhynchus tshawytscha*) fry diets at the time of the experiment.

Résumé : Nous avons ajouté du N et du P inorganiques dans une série de mésocosmes lotiques pour valider l'hypothèse selon laquelle la production de la chaîne alimentaire benthique dans la rivière Nechako, un cours d'eau de Colombie-Britannique à débit contrôlé, était limitée en N et en P. Les effets des ajouts de N, de P et de N + P ont été comparés à des valeurs témoins afin de déterminer les réponses du périphyton et des macroinvertébrés sur une période de 33 jours de la fin du printemps. L'accroissement du périphyton pour un ajout de N + P était plus du double que pour les témoins, et l'ajout de N seul a entraîné une augmentation modérée. Par contre, l'effet d'un ajout de P a eu relativement peu d'effet sur le périphyton. La densité des macroinvertébrés, particulièrement les chironomidés orthocladiinés et les éphéméroptères, a augmenté en cas d'ajout de N ou de N + P, mais pas en cas d'ajout de P ni dans les milieux témoins. Les taux accrus d'émergence observés chez les insectes aquatiques adultes des mésocosmes étaient conformes aux augmentations observées chez les invertébrés benthiques. Les résultats ont montré que la production benthique était limitée par les éléments nutritifs et que les réactions des algues et des invertébrés benthiques étaient étroitement liées. C'est l'azote qui était l'élément le plus limitant; pour des ajouts de N inorganique de seulement 10 $\mu g \cdot L^{-1}$, P est devenu limitant. Les invertébrés benthiques dont l'abondance a le plus augmenté correspondaient aux principales proies des alevins du saumon quinnat (*Oncorhynchus tshawytscha*) au moment de l'expérience.
[Traduit par la Rédaction]

Introduction

Growth rates and biomass of several trophic levels in large and small streams are known to increase with the addition of inorganic nutrients. Peterson et al. (1983), Perrin et al. (1987), and Bothwell (1989) showed that additions of P to P-deficient streams can increase the growth and biomass of periphytic algae. The concentration of added PO_4-P that saturates P-deficient growth can be <1 $\mu g \cdot L^{-1}$ (Bothwell 1989), but the concentration that saturates the accrual of areal biomass is higher due to diffusion effects within the algal mat (Bothwell 1989; Stevenson and Glover 1993). With the response by algae to nutrient augmentation in rivers, there can be an increase in benthic invertebrate abundance (Johnston et al. 1990; Hart and Robinson 1990), largely due to feeding on the more abundant food supply in periphyton (Hershey et al. 1988) which improves invertebrate survival (Mundie et al. 1991). These additions of nutrients that limit algal growth will favour energy transfer based on autotrophy rather than heterotrophy (Peterson et al. 1985) and can produce an increase in the biomass and yield of fish (Johnston et al. 1990; Deegan and Peterson 1992) due to increased availability of the food organisms.

Because of the potential for increased salmonid yield, nutrient addition to rivers has been considered a technique to mitigate for possible losses of fish production or habitat in large projects involving flow diversion and impoundment. In the planning for one of these projects, known as the Kemano Completion Project in British Columbia, Canada, nutrient addition was considered an option to mitigate potential impacts on chinook salmon (*Oncorhynchus tshawytscha*) from the diversion of water from the Nechako River, a large tributary of the Fraser River in British Columbia, by the Nechako Fisheries Conservation Program (NFCP). In 1952, the Kenney Dam was constructed on the Nechako River, approximately 280 km upstream of its confluence with the Fraser River (Fig. 1). Up to 60% of the flow in an average inflow year was diverted to turbines located at Kemano on the west coast of British

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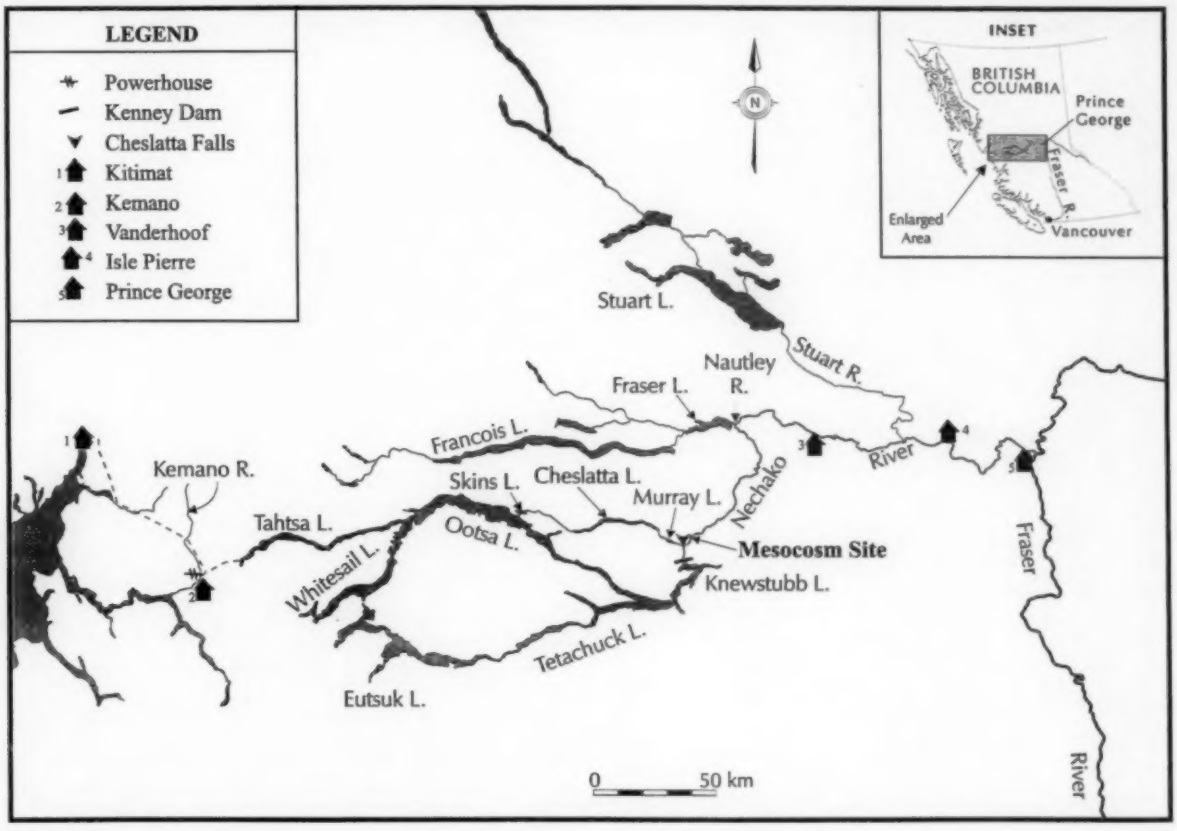
Fig. 1. Study site located within the Nechako River system.



Columbia for hydropower generation. In 1978, flows were further reduced and, beginning in 1980, were determined by legal requirements negotiated between the Department of Fisheries and Oceans (DFO) and the developer Alcan Ltd. The Kemano Completion Project was intended to divert additional water for power generation, further reducing the flow in the Nechako River. The project included the release of cool hypolimnetic water from the reservoir to the Nechako River at times of upstream migration of sockeye salmon (*Oncorhynchus nerka*) to the Stuart River, which empties into the Nechako River 80 km upstream of the confluence of the Nechako and Fraser rivers. With the release of hypolimnetic water for 1.2 months each summer, the mean daily temperature would be expected to drop by up to 6°C in the upper river. Predictions that cooler water would reduce the growth rates of chinook by 25% (Slaney et al. 1994) and that reduced flow could lead to lower recruitment rates (Bradford 1994) led the Nechako Fisheries Conservation Program to require mitigation techniques to be applied, if needed, to sustain the upper Nechako chinook population. Fertilization in combination with habitat improvement was proposed as one mitigation approach and tested using in situ trials in upper reaches during 1988–1991 (Slaney et al. 1994).

Although the addition of a growth-limiting nutrient to a river can produce a trophic response culminating in increased fish biomass (Johnston et al. 1990; Deegan and Peterson 1992), this result was not observed in the Nechako River during in situ fertilization trials. Nutrient augmentation producing 20 μg dissolved inorganic N (NO_3^--N + NH_4^+-N (DIN))·L^{-1} and 5 μg PO_4^{2-}-P·L^{-1} at full mixing in the upper river in 1989 failed to increase the size of the juvenile chinook (Slaney et al. 1994). Slaney et al. (1994) presented two hypotheses to explain the influence of other factors on this result. First, large movements of chinook fry and fingerlings in the upper Nechako River prevented the capture of fish that may have responded to any increase in availability of food in the fertilized reaches. This possibility was supported with evidence from mark and recapture studies showing that chinook fry and fingerlings did not remain in specific habitats during the summer growing season (Slaney et al. 1994). This finding meant that many fish that may have benefitted from a trophic response to fertilization may have escaped the sampling areas and were not included in the analysis of a treatment effect. The second hypothesis was that the captured chinook fry and fingerlings were growing at maximum temperature-specific rates and that fish growth was not limited by food availability. Growth modelling studies by Slaney et al. (1994) suggested that the fish were growing at close to maximum rates in May, but the rates were progressively suboptimal from June through September (Slaney et al. 1994), indicating increasing

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importance of food deficiency on growth as the summer growing season progressed. Although not explored by Slaney et al. (1994), a further hypothesis was that the fertilization did not produce an increase in abundance of invertebrates available for chinook fry and fingerlings. If the latter explanation were true, it would be unique among experiments in which responses to fertilization have been examined.

In this paper, we have examined the last hypothesis by reporting results of a mesocosm-scale experiment to quantify change in periphytic algae and benthic invertebrate composition and abundance with additions of inorganic N and P. Replicated flow-through troughs were established on the banks of upper Nechako River in which additions of N alone, P alone, and N + P were tested against an untreated control. The experiment was run at a location upstream of any other in situ testing described by Slaney et al. (1994) that was occurring in the river at the time. In a trough mesocosm, biological and chemical processes are integrated, providing invertebrates with natural food levels and environmental conditions as they are in the natural river. This simulation allows for replication and high levels of statistical power that enable considerable extrapolation of findings to larger reaches (Perrin et al. 1992). Our main objective was to quantify the change in the composition and abundance of benthic invertebrates, particularly those comprising the diet of juvenile chinook, that occurs in the Nechako River with addition of N and P alone and in combination.

Materials and methods

The existing Nechako River originates at Murray Lake, 2.6 km upstream of Cheslatta Falls (53°38′N, 124°58′W) in central British Columbia, Canada (Fig. 1). Water flows entering Murray Lake are regulated at the Skins Lake spillway which controls water discharge from Ootsa Lake, a basin of the Nechako Reservoir originally formed by impoundment of the Nechako River by construction of the Kenney Dam in 1952 (Fig. 1). Release of water at the spillway is determined by legal requirements augmented by variation in annual snowmelt and local rainfall. The mean annual flow at Cheslatta Falls is 61 $m^3 \cdot s^{-1}$ and the natural spring freshet has been shifted by flow regulation from spring to midsummer to provide cooling flows of 280–290 $m^3 \cdot s^{-1}$ to protect a major sockeye run that uses the Nechako as a migration route into the Nautley and Stuart rivers, 90 and 190 km, respectively, downstream of Cheslatta Falls (Fig. 1). The Nechako River empties into the Fraser River at Prince George, 260 km downstream of Cheslatta Falls.

The trough apparatus was installed at the river margin in a canyon approximately 4.5 km downstream from Cheslatta Falls. This site provided sufficient hydraulic head for a gravity-based water supply. Access to the site was by jet boat launched 14.5 km downstream of Cheslatta Falls. Maximum water depth across the wetted width of the Nechako River at the trough site was 0.5 m and the current velocity was about 0.6 $m^3 \cdot s^{-1}$. Mean flow of the river was regulated to 60 $m^3 \cdot s^{-1}$ for the duration of the experiment (May and June 1990). Water temperature, determined from hourly readings 19 km downstream of the Kenney Dam, was between 8 and 14°C, with temperature generally increasing throughout the experiment.

The 16 mesocosms were flow-through troughs (each 1.52 m long × 0.2 m wide) that were fabricated from 0.64-cm clear Plexiglas and assembled on a series of joists laid at the river margin. The design followed that originally reported by Mundie et al. (1991, see their fig. 1) and subsequently modified for use in chemical manipulation studies (Perrin et al. 1992; Richardson and Perrin 1994). Water and biota carried in suspension in the Nechako River were supplied via gravity through a 15.24-cm-diameter PVC pipe laid upstream over a distance of 500 m, providing a head of 0.79 m and water flow of about 8 $L \cdot s^{-1}$. The apparatus consumed a minimum of 5 $L \cdot s^{-1}$. The pipeline was fitted to a head tank and water was delivered to each trough through a standpipe assembly that was used to adjust water flow in each trough to 0.3 $L \cdot s^{-1}$.

Nutrient solutions were introduced into the upstream end of each treated trough by siphoning concentrated solutions of NH_4NO_3 and KH_2PO_4 at a rate of 0.5 $mL \cdot min^{-1}$ (calibrated daily) from 4-L glass Mariotte bottles through microbore tubing. The solutions were prepared from reagent-grade chemicals and distilled water. Nutrients were mixed with river water in a mixing chamber located at the upstream end of each trough.

Downstream of the mixing chamber was a 1.2-m section within which 2-cm-diameter drain rock was laid to a depth of 5 cm. This section provided substrata for the colonization and growth of benthic invertebrates. Downstream of the gravel was a 0.32-m section that was fitted with a sheet (1.2-cm thickness) of open-cell styrofoam (as reported by Perrin et al. 1987) that provided a surface for the sampling of periphyton biomass. The gravel and styrofoam sections were separated with a 7-cm-high baffle that produced a water depth of 7 cm over the gravel and a surface current velocity of 5 $cm \cdot s^{-1}$. Water leaving the gravel section flowed over the baffle and dropped onto the styrofoam surface. The water then flowed in a laminar pattern over the styrofoam at a depth of 1 cm.

The experiment included measures of change in periphyton accrual and species composition, as well as macroinvertebrate density, drift rate, and adult emergence as a function of nutrient augmentation. Four conditions were additions of DIN (NO_3–N + NH_4+-N) to achieve a fully mixed concentration of 10 $\mu g \cdot L^{-1}$, soluble reactive P (SRP) at 5 $\mu g \cdot L^{-1}$, and the combination of N and P contrasted with a control having no nutrient additions. Although these nutrient doses were lower than those reported in other tests of nutrient deficiency in streams (Peterson et al. 1983; Mundie et al. 1991; Slaney et al. 1994), they do exceed levels known to saturate both N- and P-limited growth of periphyton in rivers (Bothwell 1989, 1992). Each of the three treatments and the control were replicated four times producing a layout of 16 experimental units randomly assigned to treatments. Water flows were adjusted and nutrient additions started on May 24, 1990. All flows were stopped with the completion of all sampling on June 26, 1990.

Water samples were collected weekly, filtered in the field, and analyzed for concentrations of NH_4^+, NO_3^- + NO_2^-, and SRP within 48 h of sample collection. The sum of the concentrations of NO_3^- -N + NO_2^- -N and NH_4^+ -N represented DIN. In this paper, NO_3^- -N + NO_2^- -N is simplified to NO_3^- and total ammonia (NH_3 + NH_4^+) to NH_4^+.

Periphyton accrual began on May 24 with the installation of clean styrofoam substrata. In weekly intervals up to June 26 (33 days), a 2-cm-diameter core of the styrofoam and adhering biomass was removed from each trough and frozen at –15°C for shipment to the laboratory. The biomass was analyzed for chlorophyll *a* concentration using the fluorometric procedure after extraction in 90% acetone (Holm-Hansen et al. 1965; Nusch 1980). On the final sampling day, an additional core was extracted from each substratum and preserved in Lugol's solution for taxonomic identification and enumeration. Counts of cells containing cytoplasm were made at 500× magnification in Utermohl chambers. A minimum of 100 individuals of the most abundant species and a total of 300 cells in total were counted.

Insect drift from the troughs (emigration) was collected for 24-h periods on June 11, 18, and 25. The samples were collected in buckets that had openings covered with 253-μm-mesh Nitex netting. One bucket was fitted to the end of each trough and continuously filtered all water to the 253-μm size fraction. Contents were preserved in 5% formalin. All animals were separated from the algae and other detritus using a dissecting microscope at 6.4–16× magnification. Sorted samples were preserved in 70% ethanol for counting and identification.

An insect emergence trap that was identical in design to that

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Table 1. Mean SRP concentrations ($\mu g \cdot L^{-1}$) (±1 SE in parentheses) in each treatment by week of sample collection.

Date	Control	N alone	P alone	N + P
May 31	17.5 (4.4)	10.8 (4.2)	16.3 (8.9)	15.0 (2.7)
June 7	17.8 (3.8)	10.3 (0.6)	10.3 (3.0)	12.8 (2.5)
June 14	3.0 (0.7)	1.0 (0)	2.8 (0.3)	3.5 (1.3)
June 21	2.8 (1.1)	3.8 (1.2)	6.0 (1.1)	5.8 (0.5)
June 28	3.0 (2.0)	2.0 (1.0)	5.7 (3.3)	3.0 (1.0)

Note: On all dates, NO_3^- -N and NH_4^+ -N concentrations were each $<5\ \mu g \cdot L^{-1}$.

reported by Mundie et al. (1991) covered the top of each trough. Inside each trap were two longitudinal channels into which a solution of 5% formalin plus a wetting agent was poured to a depth of 1.5 cm. Emerging insects entered the chambers and were captured in the preservative. The traps were emptied weekly.

At the end of the experiment on June 26, zoobenthos was collected by washing the entire contents of each trough into a 250-μm Nitex net that was secured to the outlet. The gravel in each trough was agitated by hand for a standard period of 5 min. Retained benthos was preserved in 10% formalin. In the laboratory, all samples were subsampled and enumerated using procedures described in Mundie et al. (1983). Briefly, the sample material was settled into a "honeycomb" of 82 glass tubes (2.2 cm in diameter, 9.5 cm high) standing in a bucket half full of water. Ten tubes were drawn at random and enumerated at 40× magnification. Statistical analyses to examine treatment effects considered the mean count from the 10 tubes as a single replicate. Total numbers by taxa were determined by extrapolating tube counts based on the proportion of area in the settlement bucket that was enumerated. Aquatic invertebrates were identified to the lowest reliable taxonomic level (usually genus) using appropriate keys.

Statistical analysis of the data were by one-way ANOVA (four treatment levels) for measures taken at the end of the experiment (benthos). For variables that were repeatedly measured (periphyton biomass, adult insect emergence, drift), we tested the effect of treatment (four levels) and time (four dates) on the variable using a two-way, nested ANOVA. The *F*-test for the treatment effect was calculated using the trough-within-treatment factor as the error term which controlled for date, but did not inflate the error term, i.e., date was a repeated measure. Significant treatment differences were tested with prior contrasts or a posteriori tests (Tukey's). Some of the data (benthic densities) were log transformed to improve homogeneity of variance.

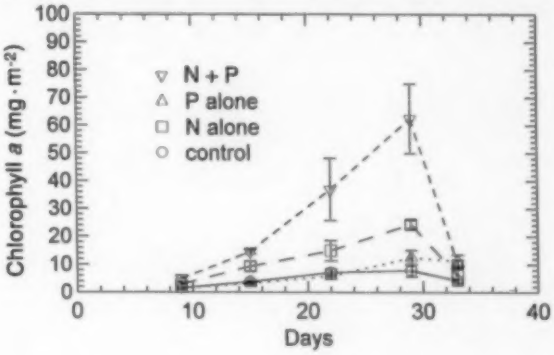
Results

Nutrients and periphyton

Concentrations of SRP in trough outflow water were relatively high and variable early in the experiment (May 31 and June 7), but they declined by June 14 (Table 1). On all dates, concentrations of NO_3^- and NH_4^+ were each $<5\ \mu g\ N \cdot L^{-1}$ in all troughs. With the exception of samples from June 21, SRP concentrations measured in troughs receiving the addition of N alone were lower than those in the control troughs.

Molar N:P supply ratios could not be determined with accuracy because of the inability to detect concentrations of the inorganic N species. If we assign the combined detection limit of $5\ \mu g\ N \cdot L^{-1}$ for each of NO_3^- and NH_4^+, to provide an assigned value of $10\ \mu g \cdot L^{-1}$, the molar N:P supply ratios ranged from 1.2 (control on June 7) to 10.1 (N alone troughs on June 14). Actual supply ratios were probably higher than these

Fig. 2. Time course changes in chlorophyll *a* concentrations (±1 SE) with additions of N + P, P alone at $5\ \mu g \cdot L^{-1}$, and N alone at $10\ \mu g \cdot L^{-1}$ in the trough mesocosms in the Nechako River.



values, but even so, they were in a range where most algal species could be limited by N and fewer by P based on optimum N:P supply ratios reported by Rhee and Gotham (1980) and Healey (1985). In the control troughs, these approximate ratios ranged from 1.3 measured on June 7 to 7.9 measured on June 21. These values are also in the range where the growth of most taxa would be expected to be potentially limited by N.

There was a significant periphyton chlorophyll *a* response to the nutrient additions (repeated measures ANOVA, $F_{3,12} = 94.4$, $p < 0.0001$; Fig. 2). The N + P addition produced a peak chlorophyll *a* concentration of $64\ mg \cdot m^{-2}$, which was greater than twice that of the response to N addition and greater than the P additions and controls. The N + P addition produced a significant increase in chlorophyll *a* concentration over the other two treatments and control (all with $p < 0.0001$). The greater chlorophyll *a* concentration with N + P versus N alone indicates that accrual was limited by P with N added alone. Addition of N alone produced a significant increase in periphyton accrual over P addition ($p < 0.03$) and the control ($p = 0.002$). There was no significant difference in chlorophyll *a* concentration between the P addition and control ($p > 0.5$).

After 29 days of biomass accrual, sloughing occurred in all troughs producing a large decline in areal biomass, particularly in treatments receiving N and N + P additions (Fig. 2) despite continuous enrichment. Periphyton chlorophyll *a* concentration in the N + P treatment declined by 83% within 4 days, and in troughs receiving only N additions, it declined by an average of 75% over the same period. In comparison, chlorophyll *a* concentration in the troughs receiving P declined by only 10% over the same period.

The relative composition of periphyton assemblages was similar for all treatments. Diatoms were the most common algae in all troughs, making up 80–90% of the algal abundance (Table 2). Chlorophytes represented 10–20% of the communities and the cyanophyte *Anabaena* sp. represented only 0.4–0.5% of communities across all treatments. *Synedra* sp. and *Fragilaria* sp. were the most common diatom taxa, representing 57.5–62.5% and 11.8–15.8% of the diatom component, respectively.

Insect emergence

The most common taxon among the emergent adult insects

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were the midges (Chironomidae) which constituted 60–76% of all adults caught in the emergence traps (Table 3). Other taxa represented in the emergence trap collections were caddisflies (Trichoptera, 12–26%), stoneflies (Plecoptera, 4–8%), mayflies (Ephemeroptera, 2–3.5%), and a few rarely encountered taxa (Table 3). The chironomid adults were not identified beyond family. Trichoptera were primarily Hydroptilidae and Rhyacophilidae, the stoneflies were mostly Chloroperlidae and Perlodidae, and mayflies were primarily represented by the genera *Baetis* and *Ephemerella*. Total numbers of insects per trough emerging over the 33 days of emergence collection ranged from a minimum of 148 in the control to 238 in troughs receiving the N additions (Table 3).

Total numbers of emerging insects increased severalfold through time ($p < 0.002$; Fig. 3) across all treatments (no significant date by treatment interaction, $p > 0.30$). The F-test for a treatment effect was significant (repeated measures ANOVA, $p < 0.04$), and the addition of N alone produced more animals than the control ($p < 0.02$), as did the addition of N + P ($p < 0.03$). The addition of P alone did not result in more insects ($p > 0.5$) and the addition of P with N did not produce more insects than that from N alone ($p > 0.7$). Chironomids were the most common taxonomic group of the emerging adults, and the results for total numbers were heavily weighted by this group. Analysis of the chironomid data alone showed similar results. Date effects were significant ($p < 0.001$) and the date-by-treatment interaction was not significant ($p > 0.5$). N additions produced more animals than the control ($p < 0.005$) and there was no effect of P added alone ($p > 0.1$).

There was no change in taxonomic richness in the emergence with treatment (repeated measures ANOVA, $p > 0.7$; Table 4). Although the control had fewest taxa after the first week of nutrient additions, it supported numbers of taxa that were within the range found in the troughs receiving the nutrients. For all treatments, however, there was a consistent and significant ($p < 0.001$) increase in richness through time, reaching an average of 8.3 taxa in the troughs receiving both N and P additions.

Benthos

Chironomid larvae were the most common insect group found in the mesocosm, with relative abundance of about 80% in control troughs to 85% in troughs receiving additions of N alone (Table 5). Chironomidae numbers were dominated by the subfamily Orthocladiinae, with lesser numbers of Tanypodinae and Tanytarsini. Pupal chironomids were also enumerated, but they were not identified beyond family. The mayflies represented 14.2% (N alone) to 18.3% (control) of the total numbers and mainly included *Baetis* sp., *Ephemerella* sp., and *Serratella* sp., the latter being most common in all treatments (Table 5). The stonefly and caddisfly numbers were only 0.1–0.5% of total benthic abundance, respectively (Table 5).

The addition of nutrients significantly increased total benthic density (Tables 5 and 6). The analysis was run independently for 11 taxa or groupings correcting for multiple comparisons (Table 6). The species tested in rank order by density for those species that occurred in numbers >0.2 animal per subsample tube or in six or more troughs are listed in Table 6. Most of the treatment response in total densities was due to significant increases in numbers of baetid mayflies (control versus N + P addition, $p = 0.005$) and particularly the orthoclad chironomids (control versus N + P addition, $p = 0.005$). The Orthocladiinae were numerically the most abundant group (Table 5) and strongly influenced treatment contrasts for the entire community. Additions of N and P significantly increased total insect densities 1.73-fold and Orthocladiinae densities by 1.77 times. Additions of N alone increased total densities by 1.54 times and Orthocladiinae densities by 1.71 times. Total densities and Orthocladiinae densities were higher with P addition but not significantly so (Tables 5 and 6). Changes in numbers of chironomid pupae were particularly striking, with densities under N addition 1.7 times those of controls and N + P additions resulting in 2.8 times higher densities. Even the addition of P increased numbers of pupae by 1.3 times. Numbers of *Baetis* sp. increased significantly only with combined N and P addition. Despite this change, numbers of *Baetis* sp. were only about 1% of the chironomid numbers and did not strongly influence treatment effects on total numbers. The mayfly *Serratella* sp. was the second most abundant taxon and treatment effects were ranked in the same order as that for the Orthocladiinae (Table 6), but variation among replicate troughs was high, resulting in no significant treatment effect. Responses by the remaining taxa generally indicated that the combination of N and P additions increased densities (Table 5), but responses to N or P alone were mixed and relative changes were small.

Table 2. Relative abundance (% of total cell counts) of periphytic algal phyla or classes and relative abundance of diatom genera (% of total counts of diatom cells) on styrofoam substrata by treatment in the experimental troughs.

Taxonomic group	Control	N added ($10\ \mu g \cdot L^{-1}$)	P added ($5\ \mu g \cdot L^{-1}$)	N + P added
Phylum or class				
Bacillariophyceae	82	88.4	79.5	86.3
Chlorophyta	16.3	10.5	20.0	12.5
Cyanophyceae	0.5	0.4	0.5	0.5
Bacillariophyceae				
Synedra sp.	62.5	58.8	57.5	61.3
Fragillaria spp.	14.3	12.3	15.8	11.8
Hannaea arcus	7.8	15.5	12.5	21.3
Tabellaria sp.	10.8	11.5	9.8	3.3
Other	4.8	2.0	4.5	2.5

Note: The "Other" group under Bacillariophyceae included *Rhopalodia gibba*, *Cymbella* sp., *Nitzschia* sp., *Gomphonema* sp., and *Melosira varians*. The chlorophytes included *Oedogonium* sp., *Closterium* sp., and *Cosmarium* sp. The only cyanophyte found was *Anabaena* sp.

Insect drift

Drift of insects, i.e., emigration from the troughs, was measured for three 24-h periods during the course of the experiment. Using the same two-way, nested ANOVA design that was used to examine time effects in the emergence, a significant increase through time in numbers of Orthocladiinae and *Serratella* sp. and total numbers of all insects in drift was found (all $p < 0.001$). Drift rates of the numerically dominant taxa (Orthocladiinae and *Serratella* sp.) and total numbers all increased between the first and last dates and most also increased between the first and second dates (Table 7). A large

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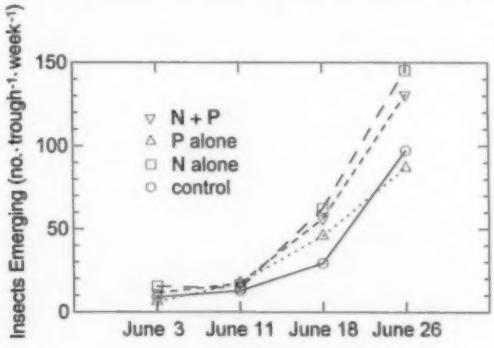
Table 3. Mean numbers by taxa and total numbers (±1 SE in parentheses) of emerging insects per trough enumerated from each treatment, and percentage of the total numbers represented by each taxonomic group.

Group	Control	N added	P added	N + P added
Ephemeroptera	5.25 (0.9), 3.5%	5.25 (1.3), 2.2%	4.75 (0.8), 3%	4.25 (1.3), 2%
Plecoptera	6.75 (1.1), 4.6%	9.75 (0.9), 4.1%	11.5 (1.2), 7.3%	19.0 (6.8), 8.8%
Trichoptera	38.75 (16.8), 26%	29.75 (1.0), 12.5%	31.25 (3.2), 20%	34.5 (9.7), 16%
Chironomidae	89.5 (22.7), 60.4%	180.5 (21.8), 76%	98.75 (12.3), 63%	147.5 (9.5), 68.4%
Other Diptera	8 (0.8), 5.4%	12.5 (2.9), 5.3%	10.5 (4.3), 6.7%	10.5 (4.6), 4.9%
Total	148.25 (38.3)	237.75 (24.5)	156.75 (12.8)	215.75 (13.5)

Fig. 3. Emergence rates of total numbers of insects per trough per week by day and treatment. Error bars are excluded for clarity.



increase was found on June 26, the last day of the experiment when algal sloughing was also noticeable.

Because numbers of drifting larvae are positively related to benthos and thus increase with N and N + P additions (Table 7), daily drift rates were normalized to benthic densities, by taxon, to determine per capita drift rates. Per capita drift rates were calculated for Orthocladiinae, *Baetis* sp., *Serratella* sp., total numbers, and taxonomic richness; these were the only groups and taxa for which there were sufficient numbers of individuals to run the analysis. In all cases, there were no significant per capita treatment effects on any group (all $p > 0.09$), indicating that per capita drift of the Orthocladiinae, the mayflies, and total numbers did not change as a function of the nutrient addition.

Discussion

Addition of inorganic N, alone and in combination with P, resulted in greatly increased accrual of periphyton and increased abundance of benthic macroinvertebrates in replicated mesocosms of the Nechako River benthic community. Findings of N deficiency in river periphyton that are not influenced by nutrient pollution are usually associated with the occurrence of volcanic parent materials (e.g., Bothwell 1992; Peterson and Grimm 1992) that contain more P compared with other formations. Exposed geology of the upper Nechako River, including extensive shorezones of Cheslatta Lake and Murray Lake and the upper 10 km of river, is typical in this regard, being characterized by volcanic andesite and basalt (Tipper 1962). Weathering of this material introduces dissolved P into the river, and assimilation of the P by benthic algae would be expected to produce a relative demand for inorganic N. At the location of the trough mesocosms in the upper Nechako River,

Table 4. Mean taxonomic richness (±1 SE in parentheses) of insect emergence caught in traps above the mesocosm troughs through time for each treatment.

	June 3	June 11	June 18	June 26
Control	2.8 (0.3)	4.8 (0.5)	6.0 (0.7)	7.0 (0.7)
N added	5.3 (0.6)	4.5 (0.9)	5.3 (1.4)	6.8 (0.6)
P added	4.8 (1.1)	3.5 (0.5)	5.8 (0.8)	7.0 (0.9)
N + P added	4.3 (1.1)	5.5 (0.9)	6.5 (0.9)	8.3 (1.0)

the unamended molar N:P supply ratio was estimated to be not less than 1.25 at high SRP concentrations in early June and 7.9 at the lower SRP concentrations in late June. The addition of N produced a greater periphyton biomass response than the addition of P, thereby showing that at the low ambient N:P supply ratios, accrual of the periphyton community was primarily N limited, a finding that was consistent with results of earlier tests of nutrient limitation in the upper Nechako River (Slaney et al. 1994). Unamended concentrations of soluble P were also low, however, and a further periphyton biomass response to the addition of P with N augmentation at only 10 $\mu g \cdot L^{-1}$ was evidence of limitation by P when N was added. If P were in surplus, the addition of P would not have produced the further response in biomass accrual.

Although N limitation is often associated with relatively high concentrations of soluble P and the occurrence of algae that can fix N, our data show that where both N and P concentrations were extremely low, N-fixers were not favoured. This finding is similar to that reported by Suttle and Harrison (1988) for an oligotrophic coastal lake. They suggested that because many N-fixers are poor competitors for available P compared with many other taxa, particularly diatoms, there is inadequate P available for N-fixers to grow at rapid rates when both N and P are present in very low supply. The same rationale may be applied to the Nechako River. Diatoms were the most abundant taxonomic group in all treatments in the mesocosm, and with low N and P concentrations even in the troughs receiving nutrient additions, they remained the most abundant group of the periphyton community. Suboptimal concentrations of available P for N-fixers may have been one factor preventing those taxa from becoming abundant at N-limited conditions.

Comparison of measured SRP and DIN concentrations with those known in the nutrient additions indicated that the periphyton were able to affect concentrations of SRP and DIN in water passing through the troughs. The depletion of P with additions of N (Table 1) was an effect likely due to P demand from the periphyton growth response to the added N. The same effect was noted during fertilization of the Nechako mainstem in 1989 (Slaney et al. 1994), when depletion of the added

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Table 5. Mean densities of groups and taxa (no.·m^{-2} extrapolated from the area of each trough) (±1 SE in parentheses) in benthos collected from the four replicate mesocosms of each treatment on June 26.

Group or taxon	Control	N added	P added	N + P added
Total numbers	14 835 (1411)	23 051 (1540)	21 113 (2627)	25 870 (2722)
Richness	11 (0.7)	10 (0.7)	9.75 (0.3)	10.5 (0.3)
Baetis sp.	37.8 (24.1)	138 (55.8)	188.8 (37.7)	252 (54.4)
Ephemerella sp.	390.1 (72.3)	176.2 (83.4)	276.8 (66.6)	541 (162.3)
Serratella sp.	2 315 (356.5)	2 982 (412.7)	2 680 (404.9)	3 637 (379.6)
Chloroperlidae	75.5 (32.5)	25.2 (14.5)	25 (14.5)	76 (43.6)
Hydropsychidae	75.5 (32.5)	101 (20.5)	25 (14.5)	88 (37.7)
Orthocladiinae	10 721 (1053)	18 297 (1308)	16 623 (2339)	18 976 (2066)
Tanypodini	302 (54.4)	125.8 (43.6)	138 (31.6)	315 (99.3)
Tanytarsini	239 (62.9)	276.8 (172.4)	365 (51.9)	377 (59.9)
Chironomid pupae	566 (113.2)	893 (172.4)	717 (148.7)	1 585 (245.3)

Note: Total numbers include rarer taxa not included in the table.

fertilizer at downstream water sampling sites could only be explained by uptake related to a growth response by periphyton downstream of nutrient additions. In the mesocosm troughs, N was added as NH_4NO_3, which would have readily dissociated into NO_3^- and NH_4^+, potentially producing a maximum added concentration of each form of only 5 μg·L^{-1}. If effects of assimilation by the periphyton are also considered with background inorganic N concentrations that were below detection, it is not surprising that NO_3^--N and NH_4^+-N were never detected.

A typical event during time-course accrual of periphyton in rivers augmented with nutrients is a massive decline in biomass shortly after a logarithmic increase in biomass has occurred (Perrin et al. 1987) despite continuous enrichment. Results from the mesocosm were consistent with this process: biomass from the N + P addition declined from a peak chlorophyll *a* concentration of 62.4 mg·m^{-2} to reach 10.5 mg·m^{-2} within the final week of the experiment. Biomass from the N addition also declined abruptly from 24.4 mg·m^{-2} to a final level of 6.2 mg·m^{-2} despite continuous enrichment. The effect was due to sloughing of the attached biomass that was induced by what appeared as progressive senescence of biomass deep within the algal mat. Bothwell (1989) and Stevenson (1990) showed that mat density can affect algal growth by producing nutrient diffusion gradients between the outside and inside of mat biomass. Stevenson and Glover (1993) also showed that the physical structure of the algal mat can interfere with the flux of nutrients within the mat and that an increase in current velocity is required to avoid declining nutrient flux as mat size increases. Because current velocity did not change with nutrient addition to our mesocosm troughs, a nutrient diffusion gradient within the mat would have increased as algal mat size increased. We hypothesize that as this process continued, nutrient limitation of biomass accrual inside the mat became severe relative to that on the outside surface, potentially leading to massive sloughing as attachments to substrata weakened and finally released due to poor or no growth inside the mat.

A consistent finding from the enumeration of emerging insects and benthos was that manipulation of N concentration had a significant indirect effect on aquatic insects, either singly or in combination with P. The positive correlation between periphyton response to nutrient addition and the insect responses suggests that insect densities are limited by the nutrient-limited production of periphyton in the Nechako River. Similar results have been found in other rivers in which nutrient augmentation has produced a functional response by grazing insects (McCormick and Stevenson 1991) and an increase in abundance of benthic invertebrates (Hart and Robinson 1990; Johnston et al. 1990; Mundie et al. 1991; Peterson et al. 1993). Together, these observations show that there can be a close coupling between periphyton biomass and benthic invertebrate abundance controlled by a limiting resource.

Insects that consume algae or fine particulate detritus were the predominant invertebrate groups in our study. Although the emerging chironomid adults were not keyed beyond family, the importance of the Orthocladiinae in both the benthos (Table 5) and drift (Table 7) suggests that the same subfamily would have been the main taxon in emergence traps. The Orthocladiinae and mayflies (e.g., *Baetis* sp.) derive their food by grazing algae or collecting particulate detritus, and diatoms are a particularly important food source for many of the taxa that responded to nutrient addition. We conclude that the increased algal biomass and detritus produced from algal growth and decomposition was the factor contributing to increased insect numbers in the mesocosm.

Previous studies of effects of enhanced food supply on aquatic insects suggest that increased numbers are partly due to improved survival of larvae (Mundie et al. 1991; Richardson and Neill 1991), and our study supports this conclusion. Change in survival rate is actually difficult to measure directly for invertebrates, and therefore, other lines of evidence are used to show effects on survival. Increases in benthic densities in experimental streams in response to food or nutrient supplementation in the absence of changes in emigration or immigration rates have been attributed to enhanced survival rate (Mundie et al. 1991; Richardson and Neill 1991). Increased patch residence time in patches with greater food resources can result in locally enhanced densities but also leads to reduced emigration rates (Kohler 1985). It is not clear whether there could be an interaction between increased benthic densities and the absence of fish predation within the mesocosms, although predaceous invertebrates were present in all troughs. Assuming that immigration rates did not differ between troughs, an increase in absolute numbers of emigrants and benthos as we observed in our experiment implies an increase in survival of immigrants.

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Table 6. Results of independent tests of orthogonal contrasts of treatment effects on total benthic density, richness, chironomid pupae, and eight taxa.

	Contrast (probability)			
Taxon or group	Control versus N addition	Control versus P addition	Control versus N + P addition	Ranking
Total numbers*	0.01	0.035	0.002	4 > 2 > 3 > 1
Richness	0.211	0.125	0.521	1 > 4 > 2 > 3
Baetis sp.*	0.114	0.024	0.005	4 > 3 > 2 > 1
Ephemerella sp.	0.097	0.405	0.415	4 > 1 > 3 > 2
Serratella sp.	0.209	0.471	0.03	4 > 2 > 3 > 1
Chloroperlidae	0.237	0.237	0.964	4 > 1 > 2 > 3
Hydropsychidae	0.504	0.211	0.761	2 > 4 > 1 > 3
Orthocladiinae*	0.007	0.026	0.005	4 > 2 > 3 > 1
Tanypodinae	0.044	0.063	0.986	4 > 1 > 3 > 2
Tanytarsini	0.764	0.214	0.182	4 > 3 > 2 > 1
Chironomid pupae*	0.168	0.485	0.003	4 > 2 > 3 > 1

Note: Critical *a* for statistical significance was 0.005 after the Bonferroni correction for multiple comparisons (not including total numbers, which is a composite measure of all the others). An asterisk following the taxon or group name indicates that at least one contrast was significant. Rank numbers are based on least-square mean densities for treatments 1 (control), 2 (N added alone), 3 (P added alone), and 4 (N + P added).

The invertebrate species that responded most to increased nutrient addition in our mesocosms are the same species that make up the diet of chinook young in the Nechako River. Stomach analyses of chinook fry captured in the same reach where the experiment was located and at the same time (Triton 1990) indicated that chironomids were the most common prey in the fry diet, followed by mayflies. Of 23 stomachs examined, the contents from 18 were predominately chironomids and six of those contained only chironomids. Given the increased abundance of chironomids and mayflies from increased algal growth, it is hypothesized that fertilization of the Nechako River can potentially lead to increased availability of important food organisms for chinook. Increased fish growth by as much as 100% resulted from nutrient addition to the Keogh River on Vancouver Island (Johnston et al. 1990) and to the Kuparuk River, Alaska (Deegan and Peterson 1992). In the Alaskan study, the response of Arctic grayling (*Thymallus arcticus*) occurred concurrently with accelerated development and increased body size of midges (Hershey et al. 1988), a common diet organism of this fish. Close linkages between autotrophic response to nutrient manipulation and fish growth have indicated that nutrient limitation in streams affects the higher trophic levels (Johnston et al. 1990; Deegan and Peterson 1992). The inability of Slaney et al. (1994) to detect this response during mainstem fertilization of the Nechako River in 1989 was probably due to factors other than food supply that affected measures of the chinook response. Slaney et al. (1994) noted that juvenile chinook were highly mobile in the upper Nechako River when the fertilization occurred, suggesting that fish movement was an important factor in confounding measurement of a fish response to fertilization.

The relatively high numbers of chironomids in the mesocosm lead to the question whether mesocosms select for this taxon in short-term experiments. The taxa found in this experiment typically occupy riffles, and their presence suggests that the mesocosm had riffle characteristics despite the low surface velocity of 5 $cm \cdot s^{-1}$. Mundie et al. (1991) suggested that uniform-sized gravel, which was used in this experiment, presents large subsurface spaces that allow rapid water movement and enhance riffle characteristics even when surface velocities are low. In lasting only 5 weeks, an important reason that chironomids were common is that many develop rapidly and have short life cycles (Stites and Benke 1989; Mundie et al. 1991), which is amenable to short-term experiments. Mundie et al. (1991) used similar troughs but allowed 4 weeks for the troughs to be colonized before nutrient addition and the experiment continued for an additional 7 weeks. Despite this longer duration, chironomids remained the most abundant taxon. Chironomids were also a major component of the benthic community during whole-river fertilization of the Kuparuk River (Hershey et al. 1988), and the Keogh River (Johnston et al. 1990). Although an experiment of much longer duration and larger scale than the present one is required to examine temporal shifts in community structure, evidence to date suggests that chironomids are functionally important in river food webs in both short-term and longer-term community development with and without nutrient manipulations. Hence, results from the mesocosm experiment are considered representative of changes in Nechako River benthos to be expected from additions of nutrients.

Two related scale issues affect the interpretation of this experiment. These are the degree to which algal herbivore dynamics are coupled and the duration of nutrient additions relative to the generation time of herbivores. One might expect the increased grazer abundances typically observed in nutrient addition experiments to limit the accrual of algal biomass. There are many examples where grazers at high densities reduce periphyton biomass (e.g., Lamberti and Resh 1983; Jacoby 1985) even with nutrient additions (McCormick and Stevenson 1991; Bothwell and Culp 1993). There are also many conditions under which such coupling of the productivities of producer and consumer may not keep pace with each other (e.g., Murdoch and McCauley 1985). For example, in the whole-river fertilization of the Keogh River (Perrin et al. 1987) and the Nechako River (Slaney et al. 1994), algal biomass increased by several times during the first few weeks of fertilization in spite of increased densities of grazing insects. In the Keogh River, the increase in algal biomass was followed

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Table 7. Mean 24-h drift rates (no. drifting·24 h^{-1}·trough^{-1}) (±1 SE in parentheses) from mesocosm troughs by day and treatment for *Serratella* sp., Orthocladiinae, and total insect numbers.

Taxon or group		June 11	June 18	June 26
Serratella sp.	Control	3.3 (0.9)	10.8 (4.5)	50.8 (6.8)
	N added	5.5 (3.3)	7.3 (2.4)	72.9 (17.5)
	P added	2.7 (1.2)	9.8 (1.9)	50.8 (12.8)
	N + P added	4 (0.7)	13.5 (3.6)	48.9 (8.2)
Orthocladiinae	Control	2.3 (0.5)	1.5 (0.9)	39.7 (10.0)
	N added	3.5 (1.8)	5.5 (2.5)	140.3 (51.1)
	P added	4 (1.2)	8.5 (4.7)	52.6 (15.8)
	N + P added	2.8 (0.6)	17.3 (1.9)	167.1 (37.9)
Total	Control	32.3 (5.0)	30.1 (6.5)	274.2 (24.3)
	N added	30 (8.4)	31.3 (2.8)	375.7 (104.0)
	P added	38.3 (4.1)	37.8 (3.1)	326.8 (51.5)
	N + P added	26.3 (8.2)	53.5 (7.2)	459.7 (68.3)

by massive sloughing of periphyton after which algal biomass never returned to high densities. This longer-term change in algal response may in part be related to a time lag effect of increasing grazer survival on the ability of grazers to crop algal biomass to low levels after an initial phase of rapid periphyton accrual (Perrin et al. 1987). These observations suggest that grazers have a slower change in density and biomass in response to change in algal biomass compared with the rate at which algae can respond to nutrient addition. These differences in rates may offset a coupling between producers and consumers in short time series of observations.

In longer-term whole river experiments, overwinter survival or other factors limiting density such as space or predation may influence relationships between the duration of fertilization and change in density and generation time of insects. Increased densities of invertebrates in response to enhanced algal production in spring and summer show that at least some species are food limited at that time of year. During the Kuparak River fertilization, algal biomass increased for the first 2 years of treatment during the ice-free period in spite of increases in insect densities, and it was only by the third year of treatment that insect densities had increased to an extent that periphyton biomass was apparently regulated by consumers (Peterson et al. 1993). In the Keogh River study, this control of periphyton biomass by invertebrates was not observed despite several years of summer-only fertilization (Johnston et al. 1990). Reasons for these differences most likely relate to river-specific conditions limiting consumer density at times of the year when fertilizer is not added. In the Alaskan study, nutrient addition lasted for most of the ice-free period. The benthic community was then frozen in place for the winter period which is potentially important for maintaining densities into the following spring (Peterson et al. 1993). In the Keogh River, increases in invertebrate densities during fertilization in spring and summer may have been offset by high flows in winter which are capable of moving bedload (Johnston et al. 1990). It can be hypothesized that these winter flows act as a reset mechanism that inhibits carryover of responses from fertilization to subsequent years. While the present mesocosm-scale experiment has revealed evidence of "bottom-up" control of fish food organism abundance in the Nechako River during short-term fertilization, these comparisons of long-term responses to fertilization in other rivers indicate that larger spatial- and temporal-scale experiments are necessary to determine actual change in availability of food organisms for fish and to determine change in the coupling between producers and consumers in a system of increased productivity.

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Nutrient limitation and herbivory: processes influenced by bleached kraft pulp mill effluent

Monique G. Dubé, Joseph M. Culp, and Garry J. Scrimgeour

Abstract: Effects of nutrient enrichment from bleached kraft pulp mill effluent (BKPME) on water chemistry, nutrient limitation status, periphyton biomass, and Orthocladiinae communities were investigated in the Thompson River, Canada, 1991–1994. Spatial differences in phosphorus (SRP) concentrations occurred downstream of a pulp mill in the winter when river flows and effluent dilution were low. Concentrations of SRP were twofold higher at the near-field site (Walhachin) compared with the far-field site (Spences Bridge), resulting in a doubling of mean periphyton accrual at Walhachin (25 $\mu g \cdot cm^{-2}$) in the winter. Individual orthoclads were 1–2 $\mu g \cdot cm^{-2}$ heavier at Walhachin in the winter compared with Spences Bridge, suggesting that nutrient enrichment from BKPME affected biomass at multiple trophic levels. Nutrient diffusing substrata (NDS) deployed at Walhachin showed that periphyton and chironomid biomass and chironomid density were not limited by P availability in the winter when BKPME concentrations were high but were P limited in the autumn when effluent concentrations were low. Algal biomass was not higher on malathion-enriched substrata in the winter compared with the autumn, suggesting that grazers were unable to reduce algal biomass in the winter when BKPME concentrations were high and periphyton growth was not P limited.

Résumé : Nous avons étudié, entre 1991 et 1994, les répercussions de l'enrichissement en éléments nutritifs provenant des effluents d'une usine de pâte kraft blanchie sur la chimie de l'eau, les limitations des éléments nutritifs, la biomasse du périphyton et les communautés d'orthocladiinés dans la rivière Thompson au Canada. Nous avons observé des différences spatiales dans les concentrations de phosphore en aval d'une usine de pâte en hiver, au moment où les débits sont bas et la dilution des effluents est faible. Ces concentrations étaient deux fois plus élevées au site le plus rapproché de l'usine (Walhachin) qu'à l'autre site (Spences Bridge), ce qui a entraîné un doublement hivernal de l'accroissement moyen du périphyton à Walhachin (25 $\mu g \cdot cm^{-2}$). En hiver, les orthocladiinés étaient individuellement plus lourds de 1–2 $\mu g \cdot cm^{-2}$ à Walhachin qu'à Spences Bridge, ce qui semble indiquer que l'enrichissement en éléments nutritifs lié aux effluents d'une usine de pâte kraft blanchie a eu un effet sur la biomasse à divers niveaux trophiques. Le dispositif de diffusion d'éléments nutritifs installé à Walhachin a révélé que la biomasse du périphyton et des chironomidés et la densité des chironomidés n'étaient pas limitées par la disponibilité en phosphore durant l'hiver au moment où les concentrations d'effluents étaient élevées, mais qu'elles étaient limitées en P en automne, lorsque les concentrations d'effluents sont faibles. La biomasse algale n'était pas plus élevée en hiver qu'en automne sur le substrat enrichi au malathion, ce qui indiquerait que les brouteurs sont incapables de réduire la biomasse algale en hiver lorsque les concentrations d'effluents des usines de pâte kraft blanchie sont élevées et que la croissance du périphyton n'est pas limitée par la disponibilité de P.
[Traduit par la Rédaction]

Introduction

Bleached kraft pulp mill effluent (BKPME) can enhance or inhibit growth of periphyton and benthic invertebrates because of a complex mixture of nutrients and toxicants in the effluent (Walden and Howard 1977; Bothwell and Stockner 1980; Hall et al. 1991; Lowell et al. 1995; Podemski and Culp 1996). Management of pulp mill wastes during the 1970s and 1980s focused on the inhibiting effects of high biochemical oxygen demand (BOD) and acute toxicity of resin and fatty acids and chlorinated organics (McLeay 1987; McCubbin and Folke 1992). With the advent of effective effluent treatment processes, extended pulp digestion, and chlorine dioxide substitution in bleaching cycles, acute effluent toxicity is no longer of primary concern (Solomon et al. 1993). Rather, eutrophication of receiving waters has become an important issue because BKPME contains high concentrations of nitrogen (N) and phosphorus (P) and total effluent discharges can exceed 150 000 $m^3 \cdot day^{-1}$. Studies in experimental streams have shown that nutrient enrichment from BKPME significantly increases periphyton accrual and benthic invertebrate growth (Lowell et al. 1996; Dubé and Culp 1996; Culp et al. 1996; Podemski and Culp 1996). Therefore, the potential exists for BKPME to increase primary and secondary productivity in receiving waters, thereby altering trophic dynamics, aesthetics, and recreational resources. Enrichment effects can also obscure effects of chronic contaminant toxicity to invertebrate and fish communities (Lozano and Pratt 1994; Gagnon et al. 1995).

In nutrient-limited rivers, enrichment typically results in strong multitrophic level responses (Fairchild and Lowe 1984;

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DeNicola et al. 1990; Winterbourn et al. 1992; Scrimgeour and Chambers 1996). In phosphorus-limited systems, P enrichment can increase periphyton biomass and the production and abundance of stream herbivores, such as chironomids and mayflies, which use periphytic algae as a primary food source (Perrin et al. 1987; Hershey et al. 1988; Mundie et al. 1991; Peterson et al. 1993; Slaney and Ward 1993). In some cases, algal biomass accrual can be moderated by increased rates of herbivory that crop algal mats (Sumner and McIntire 1982; Jacoby 1987; Lamberti et al. 1987; DeNicola et al. 1990; Mulholland et al. 1991; Scrimgeour et al. 1991; Hill et al. 1992). However, under conditions of extreme enrichment, algal growth responses may become saturated where nutrient additions exceed growth and maintenance requirements of the benthic community (Bothwell 1989; Lamberti et al. 1989; Peterson et al. 1993).

Eutrophication of the Thompson River, British Columbia, has been an environmental concern since the 1970s when extremely high accumulations of periphyton biomass occurred downstream of a municipal sewage treatment plant (STP) and a bleached kraft pulp mill (BKPM) (Federal–Provincial Task Force 1976). Historically, these excessive accumulations were thought to have occurred solely as the result of the effects of P enrichment, but Bothwell et al. (1992) recently hypothesized that reduced rates of herbivory caused by toxic effects of BKPME on the grazer community may have been an important contributing factor. Since the 1970s, P loadings from the municipal STP have declined dramatically because of tertiary effluent treatment and restriction of nutrient-rich effluent discharge during periods of low river flow. Nonetheless, eutrophication from BKPME remains a concern in the Thompson River system.

We determined if the discharge of BKPME into the Thompson River resulted in multitrophic level enrichment effects. We predicted that spatial gradients in phosphorus availability, periphyton biomass, and invertebrate abundance would exist downstream of the effluent discharge only during winter months when river flows are low and dilution of the effluent is minimal. Our second objective was to use nutrient-diffusing substrata (NDS) to determine if P enrichment from BKPME affected nutrient limitation of benthic communities. NDS were placed at a site located 14 km downstream of the pulp mill in the winter when P concentrations would be high and also in the autumn when P concentrations would be low. We predicted that benthic communities would respond to P enrichment from the NDS in the autumn but not in the winter. Our third objective was to quantify the importance of herbivory on periphyton biomass using diffusing substrata that differed in the presence and absence of malathion. We predicted that the effects of herbivory on regulating periphyton biomass would differ in the winter compared with the autumn.

Methods

Study site

The Thompson River (drainage area = 55 000 km^2) is formed by the confluence of the North and South Thompson Rivers near the City of Kamloops (Fig. 1). Below the confluence, the Thompson River flows into Kamloops Lake, then continues southwesterly for 100 km to its confluence with the Fraser River. The river is highly oligotrophic and the food web probably depends on autotrophic, autochthonous production (Bothwell et al. 1992; Wassenaar and Culp 1996). Periphyton communities consist predominantly of diatoms (e.g., *Fragilaria vaucheriae*, *Gomphonema olivaceum*, and *Hannaea arcus*) and the macroinvertebrate fauna comprises insect larvae (e.g., Chironomidae, Simuliidae, Ephemeroptera, Plecoptera, and Trichoptera). Chironomid larvae (Subfamily Orthocladiinae) are the most abundant invertebrate grazers. Fish communities consist of resident minnows (longnose dace (*Rhinichthys cataractae*) and sculpin (*Cottus* sp.)), mountain whitefish (*Prosopium williamsoni*), large-scale sucker (*Catostomus macrocheilus*), and several salmonid species. The Thompson River is important for anadromous fish, providing a spawning ground for pink (*Oncorhynchus gorbuscha*) and chinook (*O. tshawytscha*) salmon, a nursery area for steelhead (*O. mykiss*), and a migratory route for coho (*O. kisutch*) and sockeye (*O. nerka*) salmon.

Kamloops Lake is a long (25 km), narrow (mean width 2.1 km), and deep (mean depth 71 m) intermontane lake with a mean annual flushing time of 60 days (Carmack et al. 1979; Holmes 1994). Weyerhaeuser Canada Ltd. and the City of Kamloops discharge BKPME and municipal sewage, respectively, into the Thompson River immediately upstream of Kamloops Lake (Fig. 1). The Weyerhaeuser pulp mill produces ~1250 ADMt·day^{-1} (air-dried metric tonnes·day^{-1}) of 100% chlorine dioxide bleached pulp from softwood furnish and discharges ~150 000 m^3·day^{-1} of secondary treated effluent. The effluent treatment process consists of clarification, settling, and aeration in a stabilization basin with a 6- to 7-day retention time. Effluent loadings of nonfilterable residues (NFR) and BOD average 5600 kg·day^{-1} and 3900 kg·day^{-1}, respectively. Mean annual P loadings from the pulp mill to the Thompson River have increased almost threefold since 1992 (110 kg·day^{-1} in 1992 and 272 kg·day^{-1} in 1994) as the result of treatment basin fertilization. Nutrient loadings of total phosphorus from the STP are negligible compared with that released by the mill. The STP has tertiary phosphorus removal and minimizes effluent discharge during winter months when river flows are low and dilution is minimal. During low flows, the STP discharges ~7% (15 kg·day^{-1}) of the total phosphorus loadings from the mill (229 kg·day^{-1}).

Flows are unregulated in the Thompson River and annual hydrological patterns are remarkably consistent from year to year with discharge maxima occurring in May and June, annual minima occurring in January and February, and mean annual discharges approaching 720 m^3·s^{-1} (Fig. 2). Water temperature–density interactions between the river and the lake determine how inflow water, containing BKPME, affects physicochemical characteristics of downstream river reaches. Reverse thermal stratification occurs in the lake during the winter (December through March) and the colder, less dense river water (i.e., near 1°C) flows above the warmer (4°C) lake water exiting to the lower Thompson River with little intermixing (Carmack et al. 1979; Bothwell et al. 1992). Soluble reactive phosphorus (SRP) concentrations in the Thompson River downstream of the lake outlet are three- to six-fold higher than upstream of nutrient inputs (Bothwell 1992). From April to November, river water entering the lake is denser than the lake surface water and the nutrient-rich inflow mixes at depth within the lake. Because river flows are also higher at this time of year, effluent dilution is increased, and SRP concentrations reaching the lower Thompson River are ≤1.0 µg·L^{-1}. River temperatures measured at the lake outlet peak in August and September (16–19°C) and reach lows in February and March (0–2°C). Because of the thermal inertia of Kamloops Lake, the lower Thompson River remains ice-free during winter months.

Spatial and temporal patterns in water chemistry and periphyton and chironomid abundance

Spatial and temporal patterns in water chemistry, nutrient limitation, periphyton and chironomid abundance were determined downstream of Kamloops Lake at Walhachin (near-field site located 14 km downstream) and Spences Bridge (far-field site located 80 km downstream).

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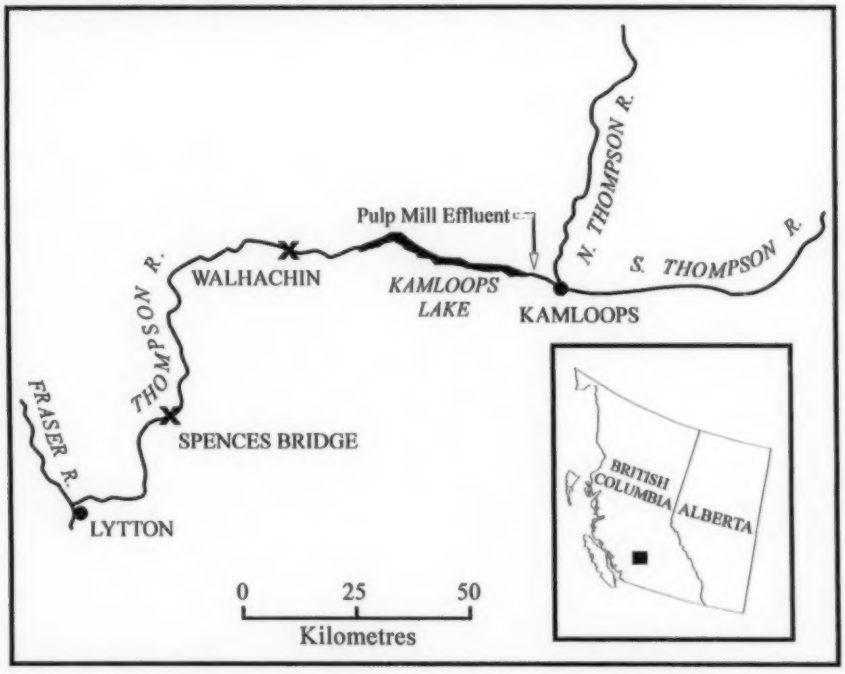
Fig. 1. Location map of the Thompson River, British Columbia.



Near-field and far-field site comparisons were conducted because an upstream reference site with habitat similar to the sites below the effluent discharge is not available. As defined by Environment Canada and the Department of Fisheries and Oceans (1993), the near-field sampling site was outside the initial effluent discharge zone and had a higher exposure to effluent than the far-field site. Water chemistry samples were collected monthly from October 1993 to March 1994 in conjunction with a more extensive sampling program (Bothwell and Culp 1993). Water samples for general water chemistry parameters were collected as grab samples in triplicate-rinsed, 1-L polyethylene bottles. Samples were kept on ice and shipped within 24 h to Zenon Environmental Laboratories, Burnaby, BC, for analysis. Samples were analysed for nutrients (nitrogen and phosphorus), total nonfilterable residues (NFR), colour, sulfate, dissolved chloride, and pH. Low-level (minimum detection concentration 1.0 $\mu g \cdot L^{-1}$) SRP samples were filtered in the field using 0.45 µm cellulose acetate filters. Averages were calculated for the winter (January, February, March) and autumn (October, November, December). When concentrations were below detection, the minimum detection limit was used to calculate averages.

Benthic samples, to quantify periphyton biomass and the biomass and density of chironomids, were collected at 2-weeks intervals from October 1991 to March 1992 and monthly between October 1993 to March 1994. On each sampling date, five rocks were randomly selected from each site and the benthos inside a circular template (9.6 cm^2) were collected. Numbers of sampled templates varied between seasons to ensure that adequate sample volumes were collected. Scrapings for periphyton abundance measurements were frozen and stored in the dark for later analyses, whereas scrapings for chironomid measurements were preserved in 10% formalin.

In the laboratory, periphyton samples were thawed, resuspended, and analysed for chlorophyll *a* (Chl *a*) ($\mu g \cdot cm^{-2}$) and ash-free dry mass (AFDM). Chl *a* was measured with a Turner Designs®, model 10 series fluorometer after a 90% ethanol extraction. Samples were not corrected for phaeopigments, which typically accounted for a small proportion of total pigment levels. Samples for AFDM were dried on aluminum dishes for 24 h, weighed, ashed at 550°C for 1 h, and reweighed.

In the laboratory, chironomid samples were sorted, identified to subfamily, counted, and individuals measured under 25× magnification with a dissecting microscope. Greater than 90% of the chironomids were Orthocladiinae, thus density and biomass estimates were restricted to this subfamily. Orthocladiinae larval body length and head width and length were measured by using a camera lucida and digitizing pad. Length measurements for each individual were converted into dry mass using length–weight regression equations previously determined for the Thompson River (Culp, J.M., National Hydrology Research Institute, 11 Innovation Blvd., Saskatoon, SK S7N 3H5, unpublished data). For each rock, total chironomid biomass ($\mu g \cdot cm^{-2}$) was calculated as the sum of the individual biomasses.

Temporal patterns in nutrient limitation

A two-factorial ANOVA design was used to test the hypothesis that periphyton biomass was affected by nutrient enrichment (presence or absence of phosphorus) and herbivory (presence or absence of malathion). Diffusing substrata experiments were conducted for 27 days in the winter (March 1993) and for 16 days in early autumn (August 1994) along a 50-m riffle at Walhachin, the closest accessible near-field, downstream location.

Diffusing substrata (DS) constructed from porous clay pots (6 cm high, 11 cm diameter, 325 mL volume) (Scrimgeour et al. 1996) were filled with a hot agar solution containing agar (20 $g \cdot L^{-1}$ in autoclaved, deionized–distilled water) and phosphorus (0.5M KH_2PO_4) and (or) malathion (0.006 M) to produce four treatments: control (C, agar only), malathion (M, agar plus malathion), phosphorus (P, agar plus phosphorus), and phosphorus + malathion (PM, agar plus phosphorus and malathion). Malathion is a cholinesterase inhibitor that can effectively inhibit grazer colonization (Winterbourn 1990; Peterson et al. 1993). DS were allowed to cool before being covered with a white,

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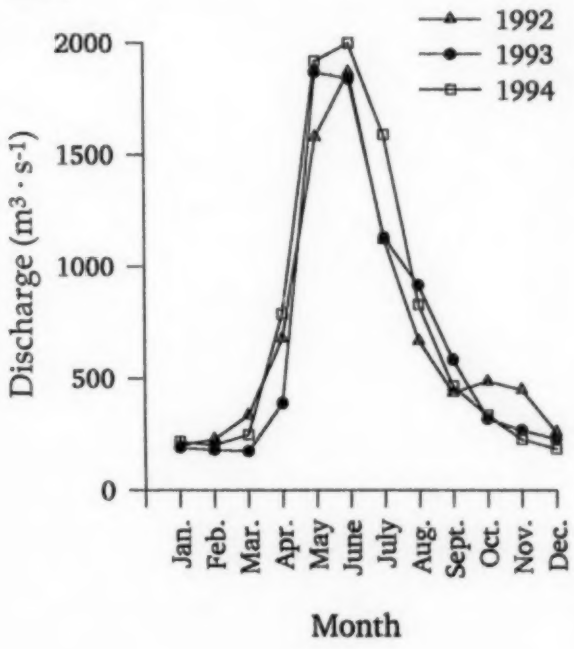
Fig. 2. Annual hydrograph ($m^3 \cdot s^{-1}$) of the Thompson River at Spences Bridge from 1992 to 1994 (data from Environment Canada).



polypropylene base (12 cm diameter, 4 mm thick), which was sealed to the DS with aquarium-safe silicon.

Forty-eight DS, representing 12 replicates of each treatment, were randomly attached to the substratum with two plastic pegs in the riffle area. Twenty-four rows of two DS were placed upstream to downstream in the riffle. DS were 0.5–1.0 m apart within each row and rows were offset by 1.5–2.0 m to minimize any potential diffusion effects from upstream treatments.

Water depth (cm) and current velocity ($cm \cdot s^{-1}$) (measured at 0.6 times the water depth) were measured at each DS at the beginning and completion of each experiment. Current velocity was measured as a 10-s average using a model 2000, portable Marsh–McBirney® flowmeter, and water temperature was recorded continuously throughout each experiment using a Ryan Instruments® thermograph.

Chironomid communities from each DS were collected using a U-net sampler (mesh size = 250 µm) (Scrimgeour et al. 1993). DS were approached from downstream to minimize disturbance and the U-net was placed over each substratum as it was lifted from the river bottom. The base of the DS and substratum immediately beneath it (to a depth of 1.5 cm) were brushed lightly to collect invertebrate fauna. Each DS was subsequently scrubbed clean with a stiff brush and the benthic sample was washed, presorted in a 250-µm sieve to remove large rocks and debris, and preserved in 10% formalin. In the laboratory, orthoclads were processed as previously described.

Periphyton biomass on the downstream facing surface of each DS was quantified by removing two circular templates (total area = 19.24 cm^2). Templates were placed into the periphyton mat and algal within the enclosed area was removed with a scalpel. Multiple templates were taken to ensure adequate sample volumes and scrapings from each template were combined, frozen immediately, and stored in the dark for later analyses. In the laboratory, the scrapings were thawed and all invertebrates were removed before samples were analysed for Chl *a* as described above.

Statistical analyses

The hypothesis that periphyton biomass and chironomid abundance were affected by season (time) and site (near-field vs. far-field) were tested with a two-factor, repeated measures ANOVA. Independent analyses were conducted for each variable in each of the sampling seasons because sampling frequencies differed between years. Linear regressions were used to test for functional relationships between SRP and Chl *a*, Chl *a* and AFDM, and Chl *a* and chironomid density and biomass (total and individual).

Effects of phosphorus (P) (presence/absence) and malathion (M) (presence/absence) enrichment of DS on periphyton biomass, total and individual chironomid biomass, and chironomid density were tested separately for the late winter and early autumn in situ experiments using two-factor ANOVA tests. Single-factor ANOVA tests were used for each experiment to determine if mean depths and velocities differed between treatments (i.e., C, M, P, PM). For this analysis, we calculated a mean depth and velocity at each DS by averaging measurements taken at the beginning (i.e., when NDS were attached to the river bottom) and completion (i.e., when NDS were retrieved) of each experiment.

For all analyses, assumptions of data normality and variance homogeneity were tested by graphically examining probability plots and residuals and by using F_{max}-tests. When test assumptions were not met, data were $\log_{10}(x + 1)$ transformed and the transformed data were reexamined. Statistical comparisons were conducted using SYSTAT (1992) software with an alpha of 0.05.

Results

Spatiotemporal patterns in water chemistry and periphyton and chironomid abundance

The measured chemical variables at Walhachin (near-field) and Spences Bridge (far-field) were similar during each season, and concentrations tended to be higher during the winter when effluent dilution was low (Table 1). The exception to this generalization was that concentrations of SRP differed markedly between seasons and sites. SRP concentrations increased between autumn 1993 to late winter 1994 at Walhachin and Spences Bridge (Fig. 3A). SRP levels at the sites were similar when the effluent was highly diluted (October and November); however, concentrations of SRP were twofold higher at Walhachin when effluent dilution was low (January and March). These results are based on limited sampling data but are consistent with a more extensive database where longitudinal patterns in SRP in 1991 showed SRP concentrations of 1–2 $\mu g \cdot L^{-1}$ at the lake outlet in the autumn compared with concentrations of 5–7 $\mu g \cdot L^{-1}$ in the winter (Fig. 3B) (Bothwell and Culp 1993). In the winter, concentrations declined from about 6.0 $\mu g \cdot L^{-1}$ at the lake outlet to 4.0 $\mu g \cdot L^{-1}$, 40 km downstream. Longitudinal reductions in SRP concentrations in the autumn were less apparent, declining from 2.0 $\mu g \cdot L^{-1}$ at the lake outlet to about 1.8 $\mu g \cdot L^{-1}$, 40 km downstream (Fig. 3B). Below 40 km, no further declines were measured as concentrations were below detection limits.

In both 1991–1992 and 1993–1994, periphyton biomass was affected by the interaction between time (month) and site (repeated measures, two-factor ANOVA on $\log_{10}(x + 1)$ data, (1991–1992, $F_{(10,70)} = 3.4$, $p = 0.001$; 1993–1994, $F_{(7,56)} = 2.3$, $p = 0.036$) and the main effects of month (1991–1992, $F_{(10,70)} = 22.0$, $p < 0.001$; 1993–1994, $F_{(7,56)} = 31.7$, $p < 0.001$) and site (1991–1992, $F_{(1,7)} = 40.0$, $p < 0.001$; 1993–1994, $F_{(1,8)} = 67.8$, $p < 0.001$) (Fig. 4). Mean Chl *a* concentrations were lowest in the autumn at both sites, increased throughout

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Table 1. Water chemistry (mean ± 1 SE) at Walhachin (near-field) and Spences Bridge (far-field) in the winter (January, February, March) and autumn (October, November, December), Thompson River, 1993–1994 (concentrations in $\mu g \cdot L^{-1}$ except where otherwise noted).

	Walhachin		Spences Bridge	
Parameter	Winter	Autumn	Autumn	Winter
N				
Total ammonia	5.7±0.7	5.0±0.0	6.3±1.3	6.3±1.3
Kjeldahl	100.0±17.3	106.7±21.9	106.7±20.3	96.7±12.0
$NO_2 + NO_3$	126.7±3.3	70.0±23.1	76.7±26.0	110.0±10.0
Total	226.7±14.5	176.7±29.6	183.3±41.8	206.7±21.9
Sulfate ($mg \cdot L^{-1}$)	16.0±2.2	10.1±2.7	11.1±2.1	16.5±2.3
Chloride ($mg \cdot L^{-1}$)	1.9±0.3	1.0±0.2	1.1±0.3	1.6±0.1
Colour (TAC)[a]	7.0±3.0	4.0±1.0	4.3±1.2	6.7±1.8
NFR ($mg \cdot L^{-1}$)[b]	65.3±5.9	68.7±4.4	73.3±7.1	72.0±7.2
pH (pH units)	7.6±0.2	7.8±0.1	7.7±0.2	7.7±0.2

[a]TAC = total absorbance colour.
[b]NFR = nonfilterable residues.

the winter where maxima were reached in January and February, and declined before the annual freshet. Spatial differences in Chl *a* were most obvious during winter months when Chl *a* was significantly greater at Walhachin than at Spences Bridge. Seasonal and spatial differences in Chl *a* were representative of differences in AFDM as a significant predictive relationship was found between Chl *a* and AFDM collected at both sites from October 1991 to March 1994 ($\log(\text{AFDM} + 1) = 0.02$ Chl $a + 7.64$, $F_{(1,187)} = 101.6$, $p < 0.001$, $r^2 = 0.40$).

Seasonal and spatial differences in chironomid density and biomass were also evident in the Thompson River (Fig. 5). Orthocladiinae density was affected by month (repeated measures, two-factor ANOVA on $\log_{10}(x + 1)$ data: 1991–1992, $F_{(10,80)} = 8.1$, $p < 0.001$; 1993–1994, $F_{(7,49)} = 14.5$, $p < 0.001$) and site (1991–1992, $F_{(1,8)} = 103.5$, $p < 0.001$; 1993–1994, $F_{(1,7)} = 81.0$, $p < 0.001$) (Fig. 5A). The interaction between the main effects was also significant in 1991–1992 ($F_{(10,80)} = 3.7$, $p < 0.001$) and in 1993–1994 ($F_{(7,49)} = 10.4$, $p < 0.001$). Seasonal trends were similar to those described for periphyton biomass as densities tended to be lower in early autumn than in late winter. Spatial trends in chironomid density were inverse to those in periphyton biomass with significantly lower densities observed at Walhachin in both years.

Total chironomid biomass was also affected by the interaction between month and site in 1991–1992 (repeated measures, two-factor ANOVA on $\log_{10}(x + 1)$ data: $F_{(10,80)} = 4.8$, $p < 0.001$) and 1993–1994 ($F_{(7,49)} = 5.5$, $p < 0.001$), indicating that site differences were dependent on the time of year (Fig. 5B). Total chironomid biomass was lower in the autumn and increased throughout the winter (1991–1992, $F_{(10,80)} = 14.3$, $p < 0.001$; 1993–1994, $F_{(7,49)} = 11.3$, $p < 0.001$). Spatial differences in total chironomid biomass also occurred between Walhachin and Spences Bridge but were not consistent between the years with sites being different in 1991–1992 ($F_{(1,8)} = 11.9$, $p = 0.009$) but not in 1993–1994 ($F_{(1,7)} = 5.0$, $p = 0.061$).

Individual chironomid biomass was low in the autumn of 1991 and 1993 and increased significantly throughout the winter with average annual maxima occurring after December (repeated measures, two-factor ANOVA on $\log_{10}(x + 1)$ data: 1991–1992, $F_{(10,80)} = 6.7$, $p < 0.001$; 1993–1994, $F_{(7,49)} = 10.3$, $p < 0.001$) (Fig. 5C). Site differences existed in 1991–1992 ($F_{(1,8)} = 32.5$, $p < 0.001$) and 1993–1994 ($F_{(1,7)} = 14.9$, $p = 0.006$) and corresponded with site differences in SRP and periphyton biomass. This indicates that larger chironomids were present at the near-field site (Walhachin), which had higher SRP concentrations and greater periphyton biomass. Site differences were more apparent from December to March after the stratification of Kamloops Lake. Interaction terms for individual biomass were significant in 1991–1992 ($F_{(10,80)} = 2.3$, $p = 0.018$), but not in 1993–1994 ($F_{(7,49)} = 1.9$, $p = 0.095$).

Relationships among phosphorus, periphyton, and chironomid abundance

Periphyton accrual (Chl *a*) was positively related to SRP concentration during the winter months of 1994 ($\log(\text{Chl } a + 1) = 0.319\text{SRP} + 2.71$, $F_{(1,6)} = 6.8$, $p = 0.04$, $r^2 = 0.53$) (Fig. 6). Linear regression relationships between chironomid density and periphyton Chl *a* at Walhachin and Spences Bridge were also significant (Table 2). Orthoclad densities were higher at Spences Bridge than at Walhachin, as indicated by differences in *y*-intercepts. Thus, for each increment in periphyton biomass, densities were greater at Spences Bridge (Fig. 7A). Total chironomid biomass (Fig. 7B) was significantly related to periphyton biomass at both sites but individual chironomid biomass (Fig. 7C) was not, suggesting that changes in total biomass were dependent on changes in the number of individual animals rather than changes in individual chironomid size.

Temporal patterns in nutrient limitation

Periphyton biomass was not affected by P enrichment or the removal of grazers in late winter (two-factor ANOVA: phosphorus, $F_{(1,37)} = 0.09$, $p = 0.76$; malathion, $F_{(1,37)} = 0.25$, $p = 0.62$) (Fig. 8). The interaction between phosphorus (P) and malathion (M) was also not significant ($F_{(1,37)} = 0.01$, $p = 0.93$). These results suggest that in the winter periphyton communities were not P limited and herbivory did not influence periphyton biomass accrual. Chironomid density and total and individual biomass were also not affected by P treatment in late winter (two-factor ANOVA: total biomass, $F_{(1,37)} = 0.03$, $p = 0.88$; individual biomass, $F_{(1,37)} = 0.03$, $p = 0.87$; density, $F_{(1,37)} = 0.59$, $p = 0.45$) (Fig. 9). Malathion reduced chironomid density (two-factor ANOVA, $F_{(1,37)} = 14.1$, $p = 0.001$)

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Fig. 3. Seasonal and spatial gradients in SRP ($\mu g \cdot L^{-1}$) at (A) Walhachin and Spences Bridge from October 1993 to March 1994 and (B) the outlet of Kamloops Lake and 40 km downstream in 1991 (after Bothwell and Culp 1993).

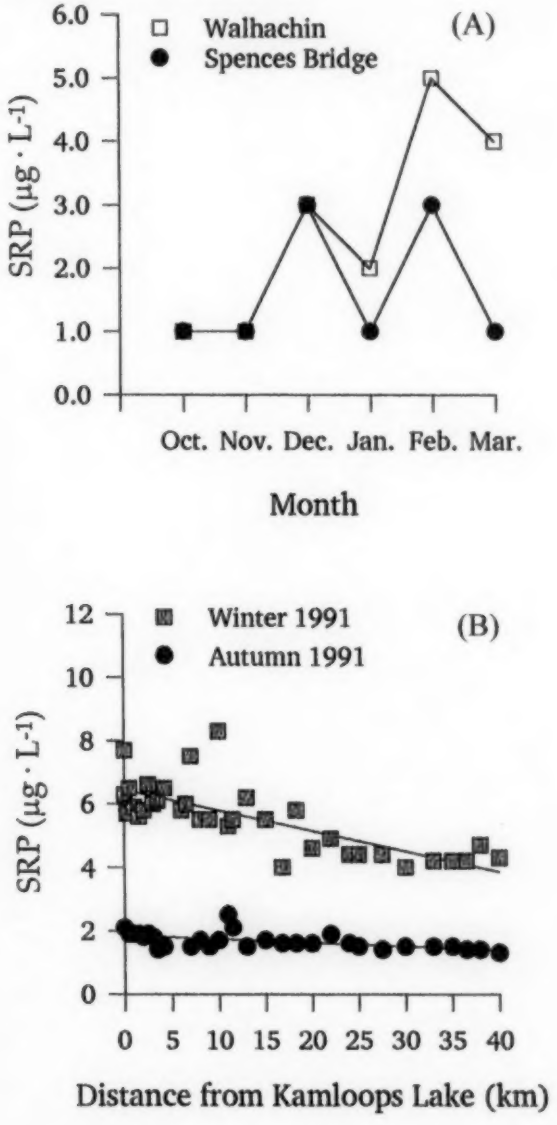
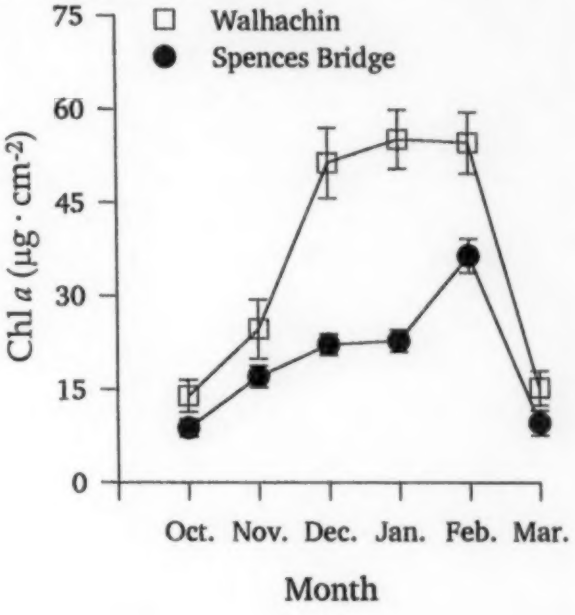


Fig. 4. Mean monthly periphyton Chl *a* ($\mu g \cdot m^{-2} \pm 1$ SE) at Walhachin and Spences Bridge. The 1991–1992 and 1993–1994 sampling years were analyzed in separate ANOVAs but were pooled for illustration purposes.



and total biomass ($F_{(1,37)} = 8.5$, $p = 0.006$) on M substrata, but the size of individual chironomids was not affected ($F_{(1,37)} = 0.2$, $p = 0.65$).

In early autumn, Chl *a* was higher on P- and M-enriched substrata than on controls without P and M. These results demonstrate that in the autumn, periphyton communities were P limited and responded to nutrient enrichment. These results also demonstrate that grazer removal resulted in increased periphyton biomass in the autumn (two-factor ANOVA: phosphorus, $F_{(1,25)} = 7.6$, $p = 0.011$; malathion, $F_{(1,25)} = 5.00$, $p = 0.03$) (Fig. 8). The interaction between the main effects was not significant ($F_{(1,25)} = 0.6$, $p = 0.45$), suggesting the effects of enrichment on periphyton growth were independent of the effects of grazing. Thus, increased periphyton biomass on P and M substrata resulted from the additive effects of phosphorus and malathion acting independently. Chironomid density and total biomass were also significantly affected by substrata treatment in the early autumn experiment (Fig. 9). Total chironomid biomass and density were higher on P-enriched substrata (two-factor ANOVA: total biomass, $F_{(1,24)} = 12.3$, $p = 0.002$; density, $F_{(1,24)} = 9.9$, $p = 0.004$). Individual chironomid biomass was also higher, though not significantly so, on P-enriched substrata ($F_{(1,25)} = 4.2$, $p = 0.051$). Chironomid abundance was significantly reduced on M substrata, demonstrating that malathion effectively reduced colonization by chironomids.

Differences in initial or final water depths and velocities among treatments were not significant in the late winter (single-factor ANOVA: depth, $F_{(3,43)} = 0.9$, $p = 0.44$; velocity, $F_{(3,43)} = 0.8$, $p = 0.49$) or autumn experiments (single-factor ANOVA: depth, $F_{(3,27)} = 2.5$, $p = 0.08$; velocity, $F_{(3,27)} = 0.2$, $p = 0.91$). However, in autumn the reduced discharge following the cessation of the freshet period resulted in some substrata becoming exposed to the air and replication decreased from 12 NDS per treatment to 7–8. During the late winter experiment, mean water temperature was 3.2 ± 0.1°C and the temperature range was 2.6–4.0°C. Mean water temperature in autumn was 18.6 ± 0.1°; minimum and maximum temperatures were 17.8°C and 19.1°C, respectively.

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Discussion

Spatiotemporal patterns in phosphorus, periphyton, and chironomids

Discharge of nutrient-rich BKPME into the Thompson River resulted in strong, seasonally dependent, multitrophic level responses by the benthic community. Seasonal gradients in SRP, periphyton and chironomid biomass, and chironomid density existed from autumn to winter and appeared to be largely defined by the hydrological regime of the river, which, in turn, determines the availability of SRP from BKPME at downstream sites. SRP concentrations were low in the autumn (1–2 $\mu g \cdot L^{-1}$) and increased to 4–6 $\mu g \cdot L^{-1}$ in the late winter. Similarly, periphyton accrual increased from 10–15 μg Chl $a \cdot cm^{-2}$ in autumn to 40–55 μg Chl $a \cdot cm^{-2}$ in late winter. Seasonal gradients in chironomid density and total and individual biomass also occurred with fewer and smaller chironomids present in the autumn and a one- to five-fold increase in chironomid number and size by late winter.

Flows in the Thompson River are unregulated, resulting in a cyclical discharge regime where flows increase to annual maxima during spring snow-melt and then decrease the rest of the year to late-winter minima. Freshet flows such as the annual Thompson River spring flood scour substrate surfaces and reduce abundance and biomass of invertebrate and epilithic benthic communities, thereby acting as important reset mechanisms for riverine biota (Biggs 1985; Scrimgeour et al. 1988; Biggs and Close 1989). Reduced discharge following the annual flood marks the initial development of a thin periphyton mat and associated chironomid communities consisting of small, early instars. During the autumn months, SRP concentrations are typically low (1–2 $\mu g \cdot L^{-1}$) (Carmack et al. 1979; Bothwell et al. 1992). Low SRP concentrations can be attributed to moderate river flows (250 and 500 $m^3 \cdot s^{-1}$), which increase dilution of the pulp mill effluent, and to mixing of lake and river water within Kamloops Lake. Results from our NDS studies show that growth of autumnal algal communities is limited by phosphorus availability, and phosphorus enrichment at this time would be expected to increase algal standing crop.

As flows decline throughout winter, the periphyton mat thickens to the point of becoming unstable by late winter. Over this period chironomid densities increase and many chironomid larvae mature through the final stages of pupal development before emerging as adults. These low flows of winter appear to enhance the accumulation of periphyton biomass and enhance the habitat for elevated chironomid abundance by creating benign physical conditions (Biggs and Close 1989; Lamberti et al. 1989; Bothwell et al. 1992). During our study, SRP concentrations reached 5–7 $\mu g \cdot L^{-1}$ at Walhachin when river flows were low. Effluent dilution is further decreased in the winter because Kamloops Lake exhibits inverse thermal stratification resulting in the river and effluent plume floating across the surface of the lake with little mixing within the lake (Carmack et al. 1979; Bothwell et al. 1992). Winter SRP levels represent a significant increase over autumn concentrations and were high enough to stimulate periphyton production because, even though cellular growth rates saturate at 1 $\mu g \cdot L^{-1}$, areal accumulated biomass continues to increase in the Thompson River with phosphorus enrichment up to at least

Fig. 5. (A) Mean monthly total density (number·m^{-2} ± 1 SE), (B) total biomass, and (C) individual biomass ($\mu g \cdot cm^{-2}$ ± 1 SE) of chironomids at Walhachin and Spences Bridge. The 1991–1992 and 1993–1994 sampling years were analyzed in separate ANOVAs but were pooled for illustration purposes.

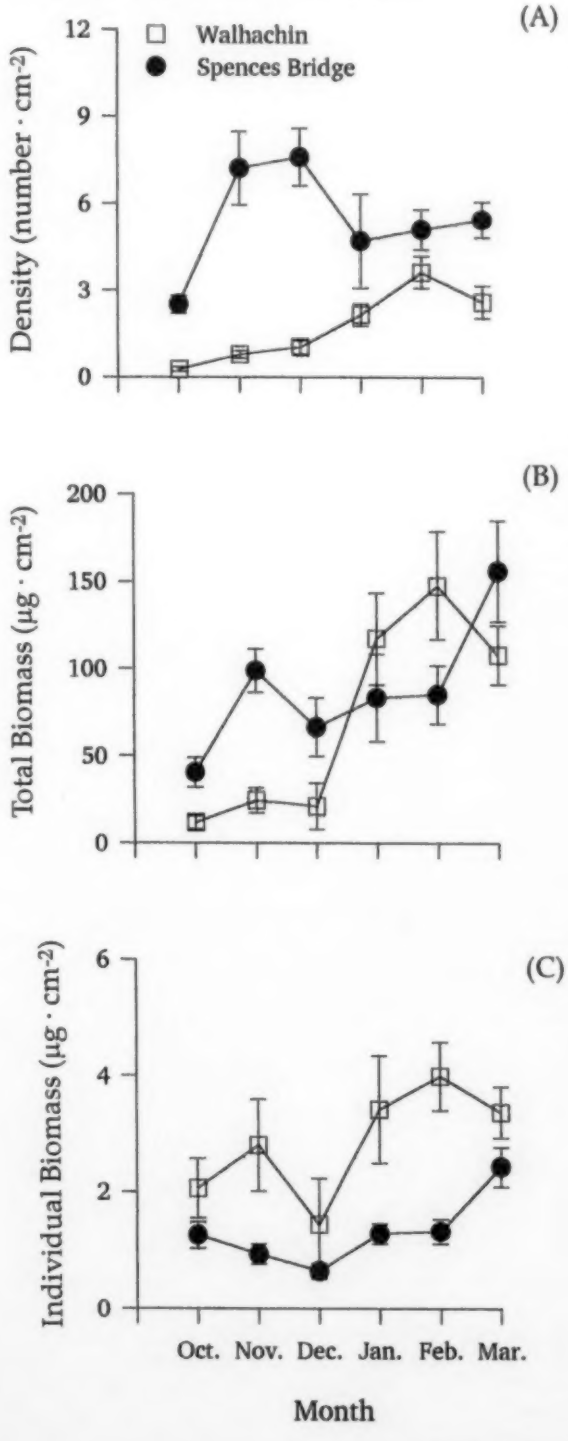

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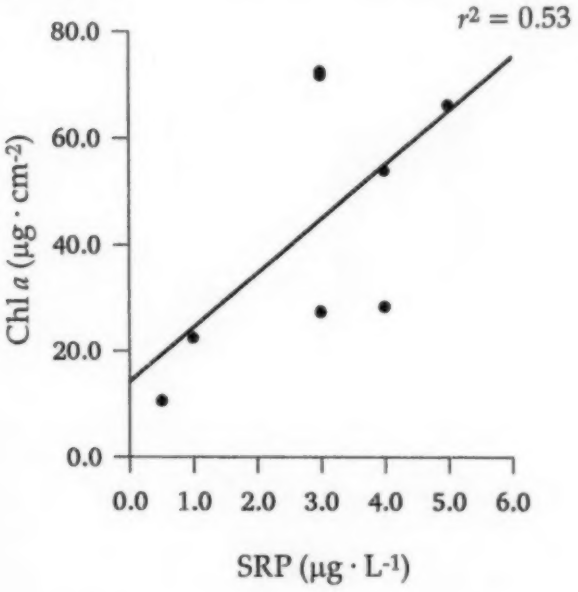
Fig. 6. Linear regression between SRP ($\mu g \cdot L^{-1}$) and periphyton Chl *a* ($\mu g \cdot cm^{-2}$) sampled at Walhachin and Spences Bridge in the winter of 1994.



30 $\mu g \cdot L^{-1}$ (Bothwell 1989; Bothwell et al. 1992). Furthermore, NDS studies illustrated that periphyton and orthoclad communities were not limited by phosphorus availability in the winter as would be expected under high SRP conditions. Our results indicate that periphyton accrual saturated when water column concentrations of SRP were 5–7 $\mu g \cdot L^{-1}$; concentrations substantially lower than those reported by Bothwell (1989) (30 $\mu g \cdot L^{-1}$). Bothwell's studies were conducted throughout the winter (47–62 days) in longitudinal troughs with styrofoam substrates where periphyton biomass became thick (20 $\mu g \cdot cm^{-2}$) with continuous P enrichment. Our studies using NDS were conducted for 27 days and the algal mat at the end of the experiments was thinner (12 $\mu g \cdot cm^{-2}$). Denser algal accumulations require higher concentrations of P to saturate and maintain growth of the whole community (Bothwell 1989).

Spatial differences in periphyton abundance and chironomid biomass existed downstream of the pulp mill during winter months and corresponded to spatial differences in SRP, thus supporting the hypothesis that nutrient-rich BKPME discharged into the Thompson River resulted in strong, seasonally dependent, multitrophic level responses. From December through March, SRP concentrations at Walhachin (near-field) were twofold higher than at Spences Bridge (far-field). This probably resulted in mean periphyton accrual at Walhachin (25 μg Chl $a \cdot cm^{-2}$) being twice as high as at Spences Bridge (52 μg Chl $a \cdot cm^{-2}$) considering the significant linear relationship ($r^2 = 0.53$) between SRP and Chl *a* in this river. Although orthoclads were 1–2 $\mu g \cdot cm^{-2}$ heavier at Walhachin than at Spences Bridge and this increased individual biomass was associated with higher SRP concentrations, we did not find a significant relationship between orthoclad size and periphyton biomass. Nevertheless, studies in experimental streams indicate that chironomid and mayfly growth are increased by

Table 2. Summary of linear regressions of orthoclad density (DEN), total biomass (TBIO), and individual biomass (IBIO) versus periphyton biomass (μg Chl $a \cdot cm^{-2}$) at Walhachin (WAL) and Spences Bridge (SB), Thompson River.

Site	Regression Equation	*F*	*p*	r^2
WAL	log DEN = 0.44 + 0.10 Chl *a*	22.9	<0.001*	0.20
SB	log DEN = 1.45 + 0.01 Chl *a*	7.2	0.009*	0.07
WAL	log TBIO = 2.62 + 0.02 Chl *a*	8.3	0.005*	0.08
SB	log TBIO = 3.71 + 0.02 Chl *a*	6.0	0.016*	0.06
WAL	log IBIO = 1.25 – 0.001 Chl *a*	0.1	0.75	0.001
SB	log IBIO = 0.83 – 0.004 Chl *a*	2.5	0.12	0.03

Note: Data were log(x + 1) transformed. For all regressions, df = 1, 92.
* = significant at alpha 0.05.

nutrient enrichment from 1% BKPME concentrations typical of winter conditions in the Thompson River (Dubé and Culp 1996; Lowell et al. 1996). Downstream differences in chironomid density were not consistent with the SRP or periphyton differences, although a significant relationship existed between density and Chl *a* at both sites. Thus, at Walhachin where SRP concentrations and periphyton biomass were high and individual chironomids were large, chironomid densities were low. It is possible that high periphyton biomass levels at Walhachin created undesirable conditions for grazing and indirectly resulted in decreased orthoclad densities or a change in orthoclad species composition. It is also possible that exposure of chironomids to BKPME resulted in a toxicity effect and decreased densities at the near-field site. Studies conducted in the Thompson River during periods of mill upsets and decreased effluent quality lend some support to this hypothesis (Dubé 1995). Additional studies are required to determine the effect of BKPME concentration on orthoclad density and the relationship between the quality and quantity of periphyton biomass and orthoclad density.

The observed spatial differences in periphyton abundance and chironomid biomass support the hypothesis that BKPME acts as a bottom-up factor, influencing periphyton growth and, possibly, size and density but not overall biomass of chironomids in the Thompson River. This conclusion is consistent with in situ fertilization studies in other phosphorus-limited rivers in British Columbia where a 5–15 $\mu g \cdot L^{-1}$ increase in phosphorus elevated periphyton Chl *a* 5- to 10-fold (Stockner and Shortreed 1978; Perrin et al. 1987; Johnston et al. 1990; Slaney and Ward 1993) and also resulted in higher insect abundance (Johnston et al. 1990).

Temporal patterns in nutrient limitation

Temporal patterns in nutrient limitation in the Thompson River are driven primarily by interactions between BKPME and the hydrological regime. Algal and chironomid communities were not expected to respond to in situ P additions in late winter because BKPME concentrations at near-field sites are high as the result of reduced effluent dilution. As predicted, Chl *a* concentrations were not significantly higher on P-enriched substrata compared with controls in late winter. Similarly, total and individual chironomid biomass and density were also not significantly different between P-enriched and control substrata. The lack of response to in situ P additions when ambient SRP concentrations are high has also been reported by Elwood et al. (1981) and Pringle et al. (1986), who

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Fig. 7. (A) Linear regressions of chironomid density (number·cm^{-2}), (B) total biomass (μg·cm^{-2} ± 1 SE), and (C) individual biomass (μg·cm^{-2}) versus periphyton Chl *a* (μg·cm^{-2}) at Walhachin and Spences Bridge from October 1991 to March 1994.

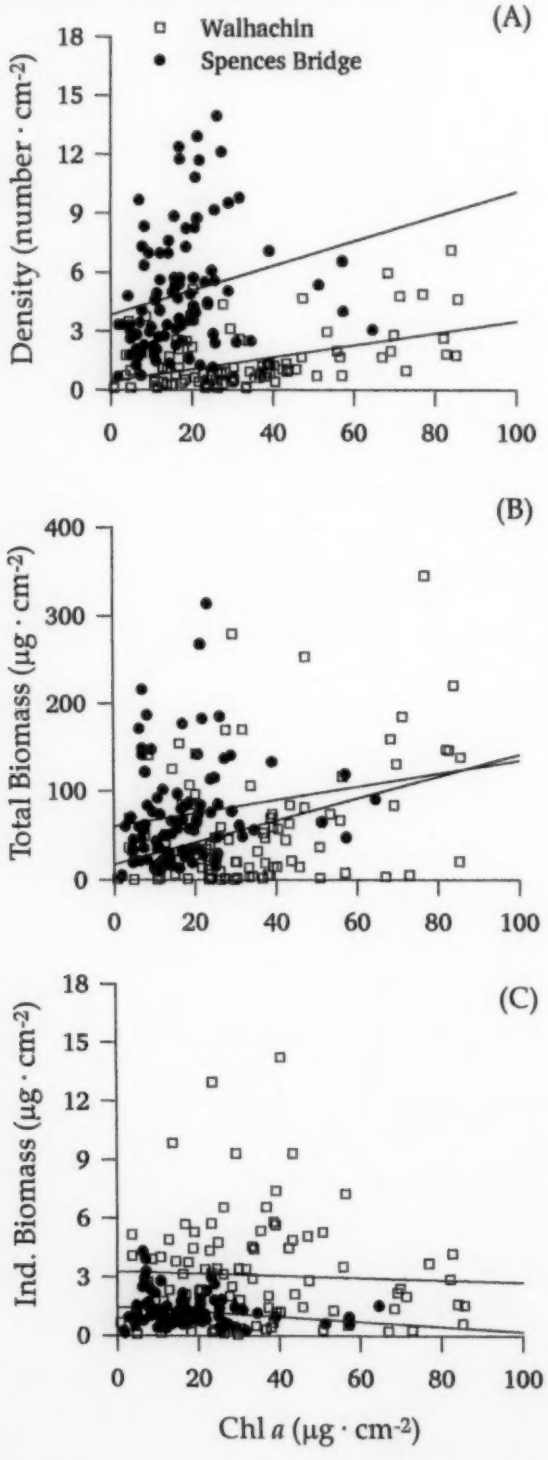
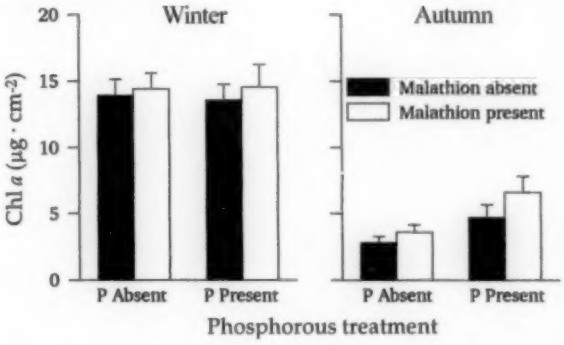


Fig. 8. Mean periphyton Chl *a* (μg·cm^{-2} ± 1 SE) on phosphorus-unenriched and phosphorus-enriched substrata in the presence and absence of malathion in the late winter and early autumn 1993–1994.



studied periphyton accrual with enrichment using NDS in temperate and tropical streams. Furthermore, Scrimgeour and Chambers (1996) found that periphyton biomass did not increase with in situ P enrichment downstream of two BKPMs discharging to the Athabasca River, Alberta. Although our data support the conclusion that BKPME resulted in increased periphyton and chironomid biomass and reduced P limitation in the winter compared with the autumn, it is not clear what role temporal differences in light and temperature may play in the response of these benthic communities to P enrichment from BKPME.

In the autumn, when high river discharge and mixing of BKPME with lake water produces low riverine SRP concentrations (Bothwell et al. 1992), periphyton Chl *a* was significantly higher on P-enriched substrata than on control substrates. Effects of in situ P enrichment were also noted in the autumn for grazers as total chironomid biomass and density were significantly higher on P-enriched substrata compared with controls. Increased periphyton biomass and invertebrate abundances to P enrichment when ambient P concentrations are low has been widely reported (Fairchild and Lowe 1984; Pringle 1987; Winterbourn 1990; Winterbourn et al. 1992). In the Athabasca River, periphyton biomass and invertebrate density were also higher on P-enriched NDS upstream of a BKPM (Scrimgeour and Chambers 1996). These results provide strong evidence that autumnal periphyton communities in the Thompson River are P limited because SRP loadings from BKPME are highly diluted. This P limitation at the primary producer level probably limits growth of chironomid grazers.

The ability of stream herbivores to reduce epilithic biomass has been widely documented (Sumner and McIntire 1982; Winterbourn and Fegley 1989; Winterbourn 1990; Hill et al. 1992; Peterson et al. 1993; Scrimgeour and Chambers 1996), and orthoclad chironomids appear to play an important seasonal role in regulating periphyton biomass in the Thompson River. In the autumn, periphyton accrual was significantly higher (approximately 25%) on malathion-enriched substrata compared with controls without malathion. Apparently, grazers can reduce algal biomass at this time when river temperatures are relatively high and periphyton growth is P limited because of low concentrations of BKPME. That the interaction term between the main effects was not significant indicates

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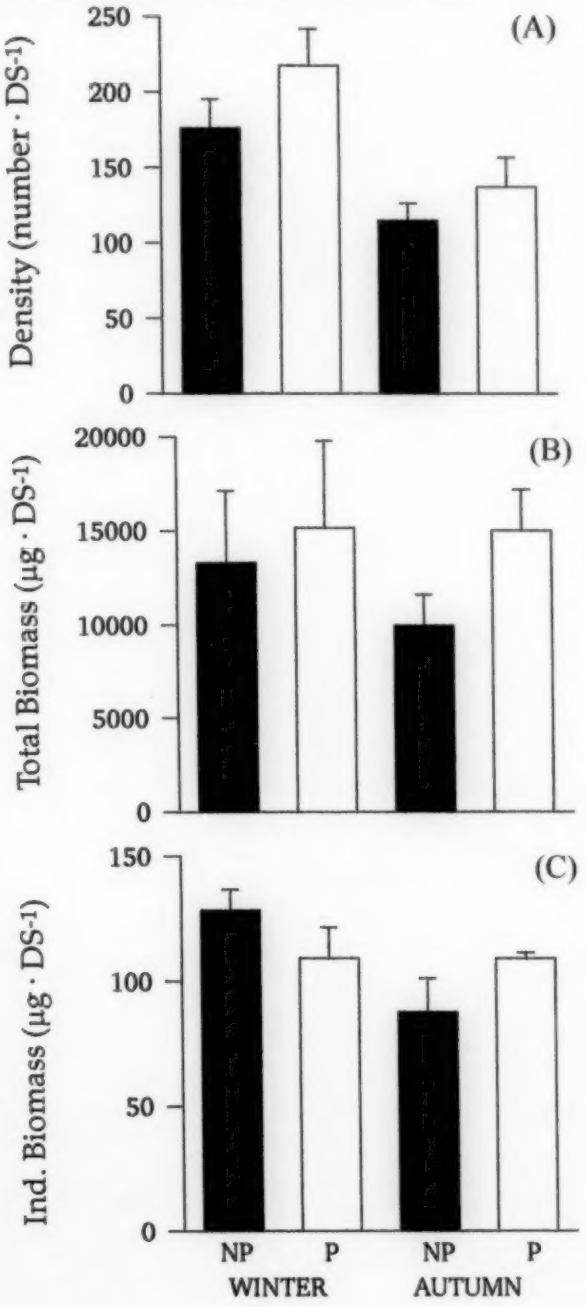
Fig. 9. (A) Mean total density (number·DS^{-1} ± 1 SE), (B) biomass, and (C) individual biomass (µg·DS^{-1} ± 1 SE) of chironomids on phosphorus-unenriched (NP) and phosphorus-enriched (P) substrata in the late winter and early autumn 1993–1994.



that the effects of P enrichment and malathion on periphyton growth are independent. Thus, in the autumn when grazers influence periphyton growth, this effect does not appear to influence the response of periphyton to P enrichment. In contrast, in late winter periphytic Chl *a* was not significantly higher on malathion-enriched substrata compared with controls. This suggests that grazers were unable to reduce algal biomass when water temperatures were relatively cold and periphyton growth was not P limited. Diffusing substrata studies using malathion treatments in the Kuparuk River, Alaska, have also found that grazers were unable to reduce algal biomass under conditions of high water column SRP (Peterson et al. 1993).

On commencement of pulp mill discharge to the Thompson River in the early 1970s, abundances of insect herbivores were dramatically reduced (Bothwell et al. 1992). Although excessive accumulations of periphyton growth downstream of the BKPM were initially attributed to P enrichment from the effluent, recent historical data suggest that some of the algal biomass increase observed may be attributed to decreased grazer abundance. This may have resulted in a trophic release and enhanced algal growth. Reductions in herbivore abundances during this period are thought to be related to upsets in effluent treatment and pulsed releases of toxic effluent (Eckstein, N., BC Environment, 1259 Dalhousie Dr., Kamloops, B.C., V2C 5Z5, personal communication). Results from our grazer control experiments do not support this "trophic release" contention because grazers influenced periphyton growth in the autumn when algal mats were thin but not in the late winter when thick algal mats accumulate. If increased effluent toxicity decreased grazer abundance during the 1970s, then our NDS grazing release treatment during the late winter should have simulated this condition. In fact, reduced grazing pressure at this time of year did not result in increased algal accrual. Thus, our new evidence suggests that excessive periphyton accumulations in the Thompson River during the 1970s largely resulted from P enrichment alone.

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The role of the zebra mussel (*Dreissena polymorpha*) in structuring macroinvertebrate communities on hard substrata

Anthony Ricciardi, Fred G. Whoriskey, and Joseph B. Rasmussen

Abstract: We examined the importance of the Eurasian zebra mussel (*Dreissena polymorpha*) in structuring macroinvertebrate communities on hard substrata in the Great Lakes – St. Lawrence River system. An experiment using artificial substrata (i.e., cement bricks with either a layer of living zebra mussels, a layer of intact empty shells that mimicked living mussels, or with no added layer) showed that macroinvertebrate abundance is enhanced in the presence of zebra mussels and that macroinvertebrate responses to physical versus biological attributes of mussel beds (e.g., spatial habitat created by clumped shells; biodeposition) vary among taxa. Moreover, densities of zebra mussels and associated epifauna have increased severalfold at various sites in the Great Lakes – St. Lawrence River system within the past decade; changes in community composition were similar to those observed in our artificial substrate experiment. Our results suggest that dense zebra mussel colonization alters macroinvertebrate communities on hard substrata by enhancing conditions for deposit-feeding organisms, small gastropods, and small predatory invertebrates, and by displacing large gastropods and certain large filterers. In the St. Lawrence River, these effects were associated with zebra mussel densities of 1500–4000 individuals/m^2, which are likely to be supported by most waterbodies in North America.

Résumé : Nous avons examiné l'importance de la moule zébrée eurasienne (*Dreissena polymorpha*) dans la structuration des communautés de macroinvertébrés sur des substrats durs dans le réseau des Grands Lacs et du Saint-Laurent. Une expérience faisant appel à des substrats artificiels (c.-à-d., des briques de ciment soit couvertes d'une couche de moules zébrées vivantes, soit portant une couche de coquilles vides intactes ressemblant à des moules vivantes, soit sans recouvrement) a révélé que l'abondance de macroinvertébrés est accrue en présence de moules zébrées, et que les taxons de macroinvertébrés varient en fonction des attributs physiques ou des attributs biologiques des gisements de moules (p. ex., habitat spatial créé par les coquilles entassées; nourriture fournie par le dépôt de matières organiques). En outre, les densités des moules zébrées et de l'épifaune associée ont augmenté plusieurs fois à divers sites du réseau des Grands Lacs et du Saint-Laurent au cours de la dernière décennie; les changements dans la composition des communautés étaient similaires à ceux que nous avons observés pendant notre expérience sur les substrats artificiels. Nos résultats font ressortir que la colonisation par les moules zébrées modifie les communautés de macroinvertébrés sur les substrats durs en stimulant les conditions favorables aux organismes déposivores, aux petits gastropodes et aux petits invertébrés prédateurs, et en déplaçant les gros gastropodes et certains filtreurs de grande taille. Dans le Saint-Laurent, ces effets étaient associés à des densités de moules zébrées de 1500–4000 individus/m^2, qui sont susceptibles d'être observées dans la plupart des masses d'eau de l'Amérique du Nord. [Traduit par la Rédaction]

Introduction

Sessile fouling organisms have been shown to play important roles in structuring benthic communities (e.g., Paine 1974; Suchanek 1979, 1986; Ban and Nelson 1987; Matsumasa 1994). On marine rocky shores, mussels form spatially complex patches (mussel beds) that provide habitat for a diverse assemblage of organisms (e.g., Tsuchiya and Nishihira 1986; Jacobi 1987; Ong Che and Morton 1992; Lintas and Seed 1994; Seed 1996) but may also reduce or displace other benthic fauna (Paine 1974; Griffiths et al. 1992; Hockey and van Erkom Schurink 1992). Dramatic changes in marine benthic communities have followed invasions by competitively dominant mussels (Rao and Rao 1975; Griffiths et al. 1992; Hockey and van Erkom Schurink 1992; Abdel-Razek et al. 1993).

The introduction of the Eurasian zebra mussel (*Dreissena polymorpha*) to the Laurentian Great Lakes in the mid-1980s (Hebert et al. 1989) added the first epifaunal mussel to North American freshwater communities, which generally have evolved without dominant macrofouling organisms. Therefore, the potential impacts of this invasion on benthic fauna and food webs have fueled much speculation (e.g., Hebert et al. 1991; Cooley 1991; Bruner et al. 1994). Although the zebra mussel has been established in Europe for almost two centuries (Stanczykowska 1977), and several European studies provide anecdotal evidence that zebra mussels influence benthic macroinvertebrate abundance (e.g., Sebestyen 1937; Dusoge 1966; Wiktor 1969; Izvekova and Lvova-Katchanova 1972; Lyakhnovich et al. 1982; Maslowski 1992; Burlakova 1995), there is little quantitative information available in the

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European literature that could be used to predict the mussel's impacts on North American benthic fauna. Within the last few years, the deleterious effects of zebra mussels on native North American freshwater mussel (Unionidae) populations have become well documented (e.g., Gillis and Mackie 1994; Schloesser and Nalepa 1994; Ricciardi et al. 1995*a*, 1996), but impacts on epifaunal communities have only recently begun to be evaluated.

During the past decade, substantial changes in the composition and abundance of benthic macroinvertebrate fauna occurred in the lower Great Lakes (Griffiths 1993; Stewart and Haynes 1994; Wisenden and Bailey 1995), where zebra mussel population densities were among the highest ever recorded (~10^5 mussels/m^2, 100-fold higher than in most European habitats; cf. Stanczykowska 1977). It is unclear whether the coincident development of dense zebra mussel populations in the region was the direct cause of these changes, but it has been suggested that the physical and biological attributes of zebra mussel beds (e.g., interstitial habitat provided by clumped mussel shells and byssal threads; filtration currents; food provided by mussel feces and pseudofeces) play important roles in structuring benthic communities (Griffiths 1993; Stewart and Haynes 1994). However, the relative importance of these attributes for macroinvertebrate assemblages on hard substrata has not been tested.

In the early 1990s, the zebra mussel expanded its range into the St. Lawrence River (Mellina and Rasmussen 1994) and subsequently invaded several other large rivers outside of the Great Lakes watershed (e.g., the Hudson, Illinois, Mississippi, Ohio, and Tennessee rivers) (Ludyanskiy et al. 1993). Because the zebra mussel is expected to become established in most North American drainages (Strayer 1991; Ramcharan et al. 1992), information on the response of St. Lawrence River benthic communities to this invasion will help predict the mussel's impacts in riverine ecosystems that have not yet been extensively colonized. In this study, we tested the hypothesis that zebra mussel colonization alters the abundance and composition of macroinvertebrates on hard substrata. Specifically, we examined the effect of zebra mussels on macroinvertebrate community development in the St. Lawrence River using deployed substrata and field surveys and evaluated the relative importance of physical and biological attributes of mussel colonies in structuring these communities.

Methods

Artificial substrate experiment

Artificial substrata (plain cement bricks individually measuring 21.5 × 11 × 6.5 cm) were used to (*i*) test whether zebra mussel beds have a significant effect on macroinvertebrate colonization and (*ii*) distinguish the relative importance of the physical structure of zebra mussel beds versus their biological characteristics in influencing macroinvertebrate abundance and composition on hard substrata. Clusters of byssally attached zebra mussels (collected from various sites in the St. Lawrence River and maintained in laboratory aquaria) were rinsed thoroughly to remove any epizoic animals and then allowed to attach to one broad (21.5 × 11 cm) surface of each of 10 bricks in aquaria; in some cases, the attachment was reinforced by the use of a thin nylon thread permanently tying the cluster to the brick. In this way, 10 bricks were covered with a layer of 128 ± 3.8 (mean ± SE) living zebra mussels (length 22.1 ± 0.6 mm). Ten additional bricks were similarly covered with a monolayer of 134 ± 3.5 intact, empty mussel shells of the same size range used in the previous treatment. For this treatment, individual mussels were eviscerated and the shells were dried at an ambient temperature of 20°C for several days; they were subsequently glued (using silicone aquarium sealant cured in air for 5 days) to one broad (21.5 × 11 cm) surface of each brick so as to mimic a living zebra mussel bed. In both treatments, >90% of this surface was covered by the zebra mussel layer, resulting in a net increase of ~60% of exposed surface area on the top face of the brick (assuming the bases of attached mussel shells to be uncolonizable surfaces). Thus, the first set of bricks (treatment 1) was intended to provide invertebrate colonizers with habitat structure (mussel shells), food (mussel biodeposits), and other advantages associated with a living mussel bed, while the second set of bricks (treatment 2) provided only habitat structure. Finally, 10 additional bricks were left bare to control for the effects of both treatments.

On May 19, 1995, the bricks were transported in buckets of water to a shallow (<2 m depth), protected littoral site on Lake St. Louis, an enlargement of the St. Lawrence River at Lachine, Que. Natural substrate at this site consisted of a dense layer of cobble on silty sand, and the local zebra mussel density was 451 ± 53 individuals/m^2 of rock surface. The 30 bricks were haphazardly deployed by a diver within an area of 10 m^2. They were placed on the cobble, with the zebra mussel cluster facing upward. After 9 weeks (on July 21, 1995), the bricks were located, carefully bagged underwater, and transported to the laboratory. All (20) of the treatment bricks and seven of the control bricks were recovered. Over 90% of zebra mussels on each of the bricks used for treatment 1 were still attached and alive at the time of recovery. Three bricks used for treatment 2 were found damaged (most of the mussel shells had become detached) and were therefore omitted from our analysis. Changes in zebra mussel numbers caused by larval settlement on the bricks during the experiment were negligible (<2% of original total). All macroinvertebrates were removed from the bricks using forceps and spray from a water bottle, washed onto a 500-µm sieve, and sorted. They were identified to at least their family level (generic level in most cases) following Merritt and Cummins (1984), Pennak (1989), and Thorp and Covich (1991). These taxa were subsequently assigned to their respective functional feeding groups (sensu Cummins 1973) based primarily on information provided by Merritt and Cummins (1984) and Thorp and Covich (1991), with the exception that all gastropods were placed within the same functional group (i.e., scrapers). The amphipod *Gammarus fasciatus* feeds on a range of food items but prefers fine particulate organic material (Delong et al. 1993) and therefore was classified as a deposit feeder.

The significance of the macroinvertebrate community response to experimental treatment was determined by multivariate analysis of variance (MANOVA) of abundance data (numbers of individuals per brick), which were $\log_{10}(x + 1)$ transformed to achieve normality and stabilize variance (Draper and Smith 1981). For individual taxa, the effects of different treatments were tested at the 0.05 level using Tukey's Studentized range test. Relationships between macroinvertebrate taxa and treatment were further analyzed by principal components analysis (PCA). The two factors that explained the most variance in the data were used as axes to ordinate the taxonomic groups that responded significantly to treatment. These and all other statistical analyses were done using SAS procedures (SAS Institute Inc. 1991).

Field survey

Macroinvertebrates were sampled at four sites that are part of the fluvial corridor of the upper St. Lawrence River (Fig. 1). Two sites were on Lake St. Louis: one was on a jetty at Lachine, Que., and the other was on the east shore of Ile Madore, an island at the confluence of the Ottawa and St. Lawrence rivers. The two remaining sites were located on the eastern end of the Soulanges Canal at Pointe-des-Cascades, Que., and on Lake St. François at Cornwall, Ont. Each of these four sites supported rich macroinvertebrate communities on

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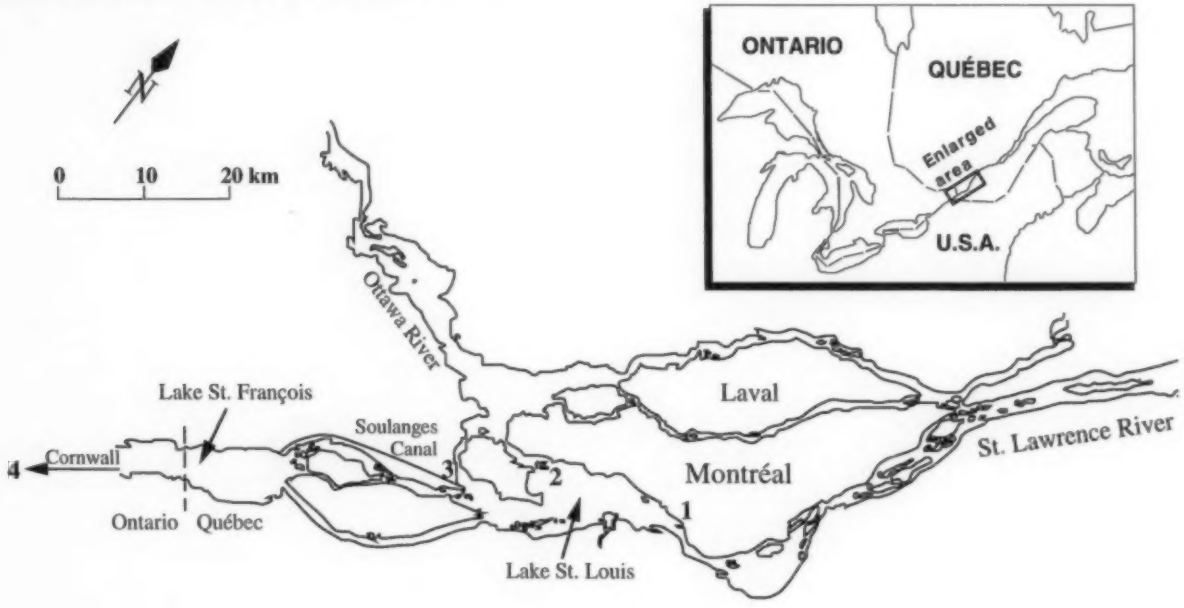
Fig. 1. Location of St. Lawrence River sampling sites (1, Lake St. Louis at Lachine; 2, Lake St. Louis at Ile Madore; 3, Soulanges Canal at Pte-des-Cascades; 4, Lake St. François at Cornwall).



abundant cobble and was invaded by zebra mussels in 1990–1991 (A. Ricciardi, personal observations). We sampled each site in September 1992, when zebra mussel densities were relatively low, and resampled them in September 1994 (September 1995 for the Lachine site) following the develpment of dense zebra mussel populations in the river (Mellina and Rasmussen 1994; A. Ricciardi and F. Whoriskey, personal observations). On each sampling occasion, 10 stones were collected randomly from <2-m depths by wading or snorkeling (except at the Soulanges Canal, where they were collected from ~6 m depth by a SCUBA diver) within an area of 10 m^2 and placed in individual plastic buckets or enamel trays. The surface area of each stone was estimated from its three orthogonal dimensions (length, width, breadth) by an equation that determines the area of an ellipsoidal shape (Dall 1979):

$$(1) \quad \text{Surface area} = \frac{\pi}{3}(\text{length} \times \text{width} + \text{length} \times \text{breadth} + \text{width} \times \text{breadth})$$

At each site, the mean surface area of stones did not vary by more than 3% between sampling occasions. The mean stone surface areas for Lachine, Ile Madore, Soulanges Canal, and Cornwall were 554, 381, 535, and 342 cm^2, respectively. All visible macroinvertebrates were removed from the stones with the aid of forceps, a bristle brush, and spray from a water bottle and were then washed onto a 500-µm sieve before being sorted and identified.

To construct a Great Lakes – St. Lawrence River data set that provided quantitative information on macroinvertebrate abundances (numbers per square metre of rock surface) before and after zebra mussel invasion, we combined our field data (Table 1) with literature data for Lake St. Clair (Griffiths 1993) and Lake Ontario (Stewart and Haynes 1994) (Table 2). Data from Wisenden and Bailey (1995) were not included in this synthesis because their experimental design (which involved the transfer of precolonized rocks between waterbodies) was not comparable with the other studies. The macroinvertebrate groups chosen a priori for analysis were three functional feeding groups (deposit feeders, scrapers, and predators) and four principal taxa (gammarid amphipods, chironomid larvae, snails, and turbellarian flatworms); these groups were chosen because they were well represented at each site in our data set before zebra mussels became abundant and are considered to be important components of epibenthic communities that have been altered by zebra mussel invasion (Dermott et al. 1993; Griffiths 1993; Stewart and Haynes 1994; Botts et al. 1996).

We tested the significance of observed changes in macroinvertebrate communities using a multivariate approach. Replicated data for St. Lawrence River sites were used to construct a robust PCA model describing macroinvertebrate community structure prior to the establishment of dense zebra mussel populations. Densities of functional or taxonomic groups sampled at the time when zebra mussel abundance was low (i.e., "before" data) for each St. Lawrence River site were normalized by $\log_{10}(x + 1)$ transformation and ordinated using PCA (SAS Institute Inc. 1991). The principal component (PC) that explained the most variance was used to generate scores from before (baseline) and "after" (post-invasion) data for each site (including Great Lakes sites; because raw replicated before data from the Great Lakes were unavailable to us, the St. Lawrence River model was applied to all sites). Each score represented a linear sum of the products of PCA loadings and macroinvertebrate densities. The differences between before and after site scores were then tested at the 0.05 level of significance using paired-comparison t tests. We excluded Ile Madore from this comparison, because it was the only site in our data set where zebra mussels did not settle in large numbers during our sampling period; the zebra mussel population density was only 73 individuals/m^2 in 1994, which is an order of magnitude lower than the lowest densities at which significant community changes associated with zebra mussel invasions have been reported. Differences between sites were not tested, as we were primarily interested in how macroinvertebrate community structure had changed over time in densely colonized areas of the Great Lakes – St. Lawrence River system. Bonferroni-corrected t tests were used on $\log_{10}$-transformed

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Table 1. Macroinvertebrate densities on rocks at St. Lawrence River sites.

	Lake St. Louis, Lachine, Que.		Lake St. Louis, Ile Madore		Lake St. François, Cornwall, Ont.		Soulanges Canal, Pte-des-Cascades, Que.	
	1992	1995	1992	1994	1992	1994	1992	1994
Mollusca								
Bivalvia								
Dreissenidae	176 (22)	3876 (96)	6.8 (3.7)	73.0 (17.3)	82.7 (14.1)	1502 (157)	504 (48)	3185 (228)
Gastropoda								
Ancylidae	—	—	—	—	—	—	35.0 (11.1)	178.5 (30.0)
Hydrobiidae	23.7 (8.4)	406.6 (38.9)	—	—	—	—	—	—
Pleuroceridae	9.4 (6.4)	0	2.5 (1.7)	6.2 (3.4)	11.3 (5.9)	8.2 (5.7)	8.4 (3.1)	1.6 (1.6)
Bithyniidae	12.8 (5.1)	42.7 (11.1)	49.0 (11.1)	82.1 (22.3)	21.5 (6.8)	36.0 (11.7)	80.4 (15.1)	223.4 (34.2)
Valvatidae	0	28.1 (10.1)	—	—	—	—	—	—
Planorbidae	16.5 (5.1)	258.4 (29.0)	25.8 (10.1)	21.7 (9.0)	7.0 (5.3)	34.0 (14.3)	7.6 (4.9)	1.6 (1.6)
Physidae	—	—	12.0 (9.3)	57.8 (26.3)	12.0 (6.3)	19.9 (10.0)	4.1 (2.7)	48.9 (14.4)
Lymnaeidae	3.6 (2.4)	1.6 (1.6)	—	—	—	—	15.4 (6.9)	25.6 (5.2)
Crustacea								
Amphipoda	77.2 (19.9)	1634 (113)	11.4 (4.8)	16.3 (7.7)	62.3 (12.1)	642.5 (64.8)	176.5 (24.5)	1097 (131)
Isopoda	5.1 (2.9)	28.4 (9.5)	6.8 (3.7)	1.6 (1.6)	—	—	9.5 (4.6)	90.3 (22.2)
Insecta								
Trichoptera								
Helicopsychidae	—	—	48.4 (7.5)	91.4 (18.8)	41.0 (9.4)	54.0 (11.3)	—	—
Leptoceridae	26.3 (10.3)	113.4 (34.6)	11.4 (5.2)	22.8 (9.3)	15.2 (6.9)	41.4 (11.8)	—	—
Polycentropodidae	—	—	—	—	19.4 (5.9)	12.9 (6.8)	13.4 (4.0)	0
Ephemeroptera								
Heptageniidae	12.8 (4.7)	57.5 (15.7)	43.6 (9.3)	29.2 (9.1)	23.3 (8.7)	61.1 (15.4)	41.3 (13.2)	105.1 (20.5)
Baetidae	57.1 (18.3)	227.1 (35.4)	—	—	—	—	—	—
Coleoptera								
Psephenidae	—	—	29.7 (8.6)	15.5 (7.1)	—	—	—	—
Elmidae	—	—	9.5 (5.1)	26.1 (11.5)	—	—	—	—
Diptera								
Chironomidae	281 (31.2)	1946 (111)	20.0 (7.3)	27.9 (7.6)	53.7 (13.4)	431.1 (37.2)	195.5 (26.3)	696.1 (92.1)
Turbellaria								
	54.1 (9.1)	624.3 (22.4)	38.5 (11.1)	72.1 (19.6)	23.2 (5.7)	104.9 (24.9)	46.7 (12.4)	332.0 (43.3)
Annelida								
Hirudinea								
Glossiphoniidae	8.0 (3.4)	75.0 (18.2)	5.8 (4.4)	18.5 (15.2)	13.4 (4.8)	49.3 (15.4)	10.7 (38)	100.4 (22.7)
Erpobdellidae	—	—	—	—	1.7 (1.7)	5.0 (3.4)	—	—
Oligochaeta								
Naididae	22.4 (11.5)	310.5 (39.0)	—	—	4.3 (3.0)	71.9 (24.7)	45.5 (16.7)	263.4 (53.3)
Spongillidae								
	34.9 (18.5)	55.7 (18.9)	—	—	—	—	35.0 (18.2)	62.2 (29.3)
Ectoprocta								
Plumatellidae	371.2 (110)	54.4 (24.8)	—	—	—	—	—	—
Total no.[a]	610 (99.9)	5753 (315)	314.4 (38.3)	489.4 (41.5)	309.3 (47.6)	1572 (179)	690.0 (85.2)	3164 (396)

Note: Values are mean numbers of individuals/m^2 of substrate (for sponges and ectoprocts, values represent colony area in cm^2/m^2 of substrate), with standard errors in parentheses.

[a] Excluding *Dreissena*, sponges, and ectoprocts.

data to test changes in the densities of zebra mussels and associated macroinvertebrates.

Results

Artificial substrate experiment

In our artificial substrate experiment, macroinvertebrate numbers varied significantly among treatments (Wilks' $\lambda = 0.0045$, $p < 0.0001$). The mean total number of invertebrates (excluding zebra mussels) on bricks declined across the three treatment levels and was about fourfold higher in the presence of live mussels than on bare substrate (Fig. 2). Individual taxa that responded to treatment (F tests, $p < 0.05$) were ordinated along two PCs that cumulatively explained 75% (PC1 = 57.6%, PC2 = 17.3%) of the standardized variance (Fig. 3). The first principal component (PC1) apparently describes an organism's affinity for mussel beds (treatments 1 and 2) compared with bare substrate (control), reflecting the general trend shown in Fig. 2 (i.e., live mussels > dead shells >> control). Groups that loaded positively on this axis (i.e., deposit feeders

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Table 2. Densities (mean no./m^2) of principal macroinvertebrate taxa and functional feeding groups at Great Lakes sites.

Site	*Dreissena*	*Gammarus*	Chironomids	Snails	Flatworms	Deposit feeders	Scrapers	Predators	Total[a]
Lake St. Clair[b]									
May 1983	0	<1	280.0	20.0	<1	260.0	20.0	240.0	800.0
June 1991	20 520	400	360.0	340.0	300.0	5440.0	340.0	460.0	6500.0
Lake Ontario[c]									
Cobble site									
Aug. 1983	0	357.8	6.0	48.1	12.0	391.5	55.3	23.7	531.0
Aug. 1992	20 773	2095.1	67.0	1311.1	29.3	3506.1	1313.4	69.5	4971.0
Reef site									
Aug. 1983	0	669.9	2.4	197.6	6.0	672.3	201.2	7.2	881.9
Aug. 1992	30 597	1524.4	12.1	363.3	25.6	1703.6	364.5	39.0	2115.8

[a] Excluding *Dreissena*.
[b] Data are from Griffiths (1993).
[c] Data are from Stewart and Haynes (1994

Fig. 2. Mean macroinvertebrate densities (± 1SE; excluding zebra mussels) after 9 weeks of colonization on experimental treatment bricks (with living mussels or with shells only) and control bricks.

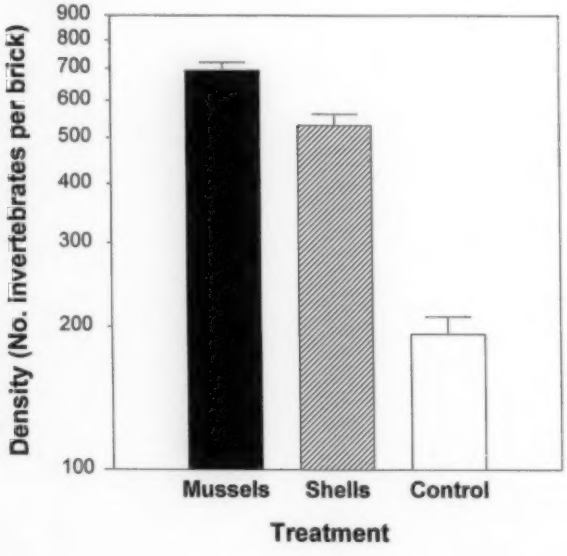
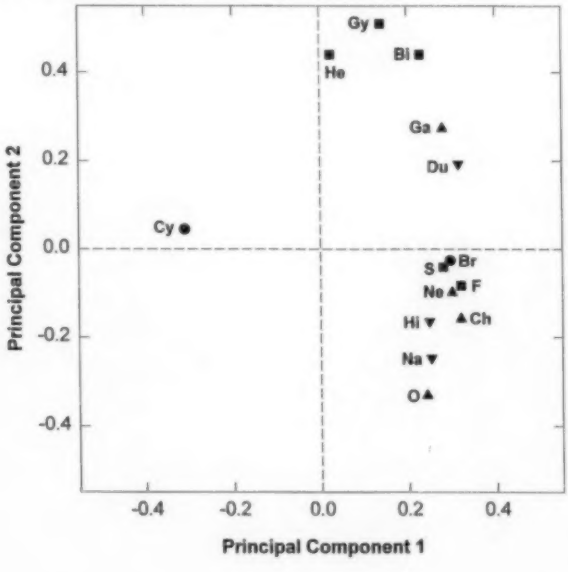


Fig. 3. Ordination of macroinvertebrate groups that responded significantly to treatment in the artificial substrate experiment. The first two principal components are shown. We interpret principal component 1 as a reflection of macroinvertebrate response to mussel-covered substrata (treatments 1 and 2) versus controls and principal component 2 as a response to substrate covered with dead shells (treatment 2) versus living mussels (treatment 1) or controls. Data are coded according to their functional feeding group: scrapers (■), deposit feeders (▲), filterers (●), and predators (▼). He, *Helicopsyche* (caddisfly); Gy, *Gyraulus* (snail); Bi, *Birgella* (snail); F, *Ferrissia* (limpet); S, *Stenonema* (mayfly); Ga, *Gammarus* (amphipod); Ne, *Nectopsyche* (caddisfly); Ch, chironomids; O, ostracods; Cy, *Cyrnellus* (caddisfly); Br, *Brachycentrus* (caddisfly); Du, *Dugesia* (flatworm); Hi, leeches; Na, naidid oligochaetes (primarily *Chaetogaster*).



and predators) colonized bricks with living zebra mussels in substantially greater densities than on control bricks (Fig. 4). *Gammarus* differed from other deposit feeders (Fig. 5) by responding only to mussel structure (aggregated shells of living or dead mussels). Predators (e.g., leeches, flatworms, and *Chaetogaster*) and commensal organisms (e.g., *Brachycentrus*) were found on live mussels in greater numbers than on dead shells or bare substrate (Fig. 5). Conversely, the large filter-feeding caddisfly *Cyrnellus* loaded negatively on the PC1 axis (Fig. 3).

The second principal component (PC2) appears to describe an organism's preference for surfaces with dead shells (treatment 2) compared with living mussel beds or bare substrate. The weak positive response by *Cyrnellus* on PC2, coupled with its strong negative response on PC1, indicates an aversion to surfaces covered with living mussels and an indifference towards dead mussel shells (Fig. 5). Most scrapers (i.e., *Helicopsyche, Gyraulus, Birgella*) loaded positively on PC2. *Helicopsyche*'s preference for surfaces with dead shells and indifference toward living mussels (Fig. 5) is refected by its

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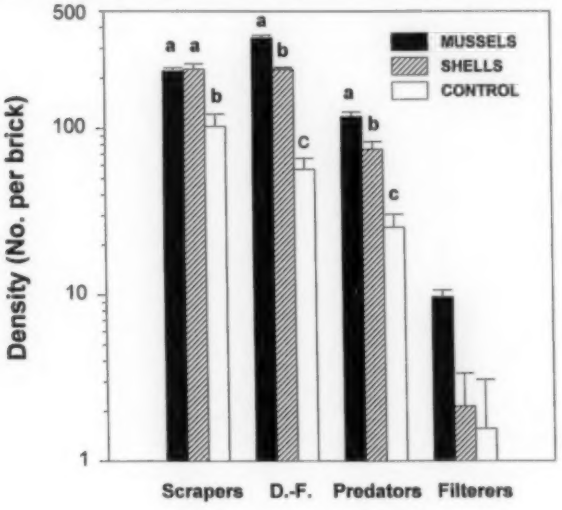
Fig. 4. Mean density (±1 SE) of functional feeding groups on treatment bricks in the artificial substrate experiment. Bars having different letters above them differ significantly (Tukey's range test).



strong positive score on PC2 and its trivial score on PC1 (Fig. 3). By contrast, deposit-feeding chironomids and ostracods were enhanced in the presence of living mussels, but did not respond to dead shells, and therefore scored negatively on PC2. The treatment response of these organisms was mirrored by their predators (Fig. 5).

In summary, almost all taxa increased in abundance on substrata covered with mussels (living or dead), and most achieved higher densities in the presence of living mussels compared with dead shells.

Field survey

Zebra mussel densities on rocks at St. Lawrence River study sites increased 14-fold, on average, over the 2–3 year period between sampling dates (Fig. 6). At every site, densities of associated macroinvertebrates increased by 1.6 to 8.4 times initial values. The smallest increase in macroinvertebrate density was observed at Ile Madore, which had the smallest zebra mussel population (73 individuals/m^2); at all other sites, macroinvertebrate density increased at least fivefold. Paired comparisons of PCA site scores for communities sampled before and after the establishment of dense zebra mussel populations at Great Lakes – St. Lawrence River sites indicate that macroinvertebrate community structure was altered substantially ($t = 8.68$, $p < 0.0003$, using taxonomic group data and a PC that accounted for 80% of the variance; $t = 5.37$, $p < 0.003$, using functional group data and a PC that accounted for 77% of the variance). The direction and magnitude of changes in dominant functional feeding groups (Fig. 7) and taxonomic groups (Fig. 8) were similar among the three St. Lawrence River sites, where final dreissenid densities were ~1500–3900 mussels/m^2, and agreed with the results of our artificial substrate experiment.

Following the establishment of dense zebra mussel populations, deposit-feeding organisms (e.g., amphipod and isopod crustaceans, chironomid larvae) dominated benthic assemblages at Great Lakes and St. Lawrence River sites. Populations of *G. fasciatus* increased dramatically, particularly in Lake St. Louis at Lachine, where their density multiplied by a factor of 20 between 1992 and 1995. Turbellarian flatworms and small gastropods also became highly abundant at most sites (Tables 1 and 2).

Only a few taxa responded negatively to zebra mussel colonization at St. Lawrence River sites. In the Soulanges Canal, larvae of the polycentropodid caddisfly *Cyrnellus* sp. were eliminated from rocks that became densely covered with zebra mussels. Large pleurocerid snails (*Elimia livescens*) declined at each of the three mussel-infested St. Lawrence River sites; they were virtually eliminated at the Lachine and Soulanges Canal sites, although smaller gastropods (hydrobiids, physids, limpets, etc.) increased substantially (Table 1). The plumatellid bryozoan *Plumatella fungosa*, which historically dominated cobble at the Lachine site, was reduced to a few small colonies on zebra mussel shells and on surrounding substrate; areal coverage by this species declined by 85%. By contrast, areal coverage by sponges (*Eunapius fragilis* and *Ephydatia muelleri*), which became abundant on mussel shells in 1994–1995, did not change significantly (Table 1).

Discussion

Comparison of field and experimental studies

Our findings strongly support the view that dense zebra mussel populations have a profound impact on the structure of macroinvertebrate communities on hard substrata, as claimed in earlier studies (e.g., Dusoge 1966; Griffiths 1993; Dermott et al. 1993; Stewart and Haynes 1994). Parallel changes in macroinvertebrate abundance and composition at Great Lakes and St. Lawrence River sites suggest that habitat differences had less influence on community structure than the presence of dense zebra mussel beds. These changes were characterized by multifold increases in macroinvertebrate densities, and the development of mussel bed communities dominated by *Gammarus*, turbellarian flatworms, and chironomid larvae.

The results of our before and after field comparisons and artificial substrate experiment were remarkably similar. The direction and magnitude of the change in abundance of deposit feeders, predators, and scrapers on rocks at Great Lakes – St. Lawrence River sites mirrors observed differences in community structure on bricks with and without zebra mussels. After 9 weeks, the treatment bricks developed mussel-associated communities that were remarkably similar to those observed at the Great Lakes – St. Lawrence River sites 4 years after colonization by zebra mussels. The mean density of living zebra mussels on the treatment bricks (1450 individuals/m^2) was 50% lower than the mean density on stones at our St. Lawrence River sites in 1994–1995 (2854 mussels/m^2). Zebra mussel densities in the St. Lawrence River were ~10-fold lower than those observed at the Great Lakes sites, yet resulted in similar changes to the macroinvertebrate community, which suggests that these changes may be better correlated with zebra mussel biomass or that they may be provoked by moderate zebra mussel densities. The zebra mussel density at Ile Madore (73 mussels/m^2) was evidently insufficient to cause significant community changes like those that occurred at the other St. Lawrence River sites. However, the

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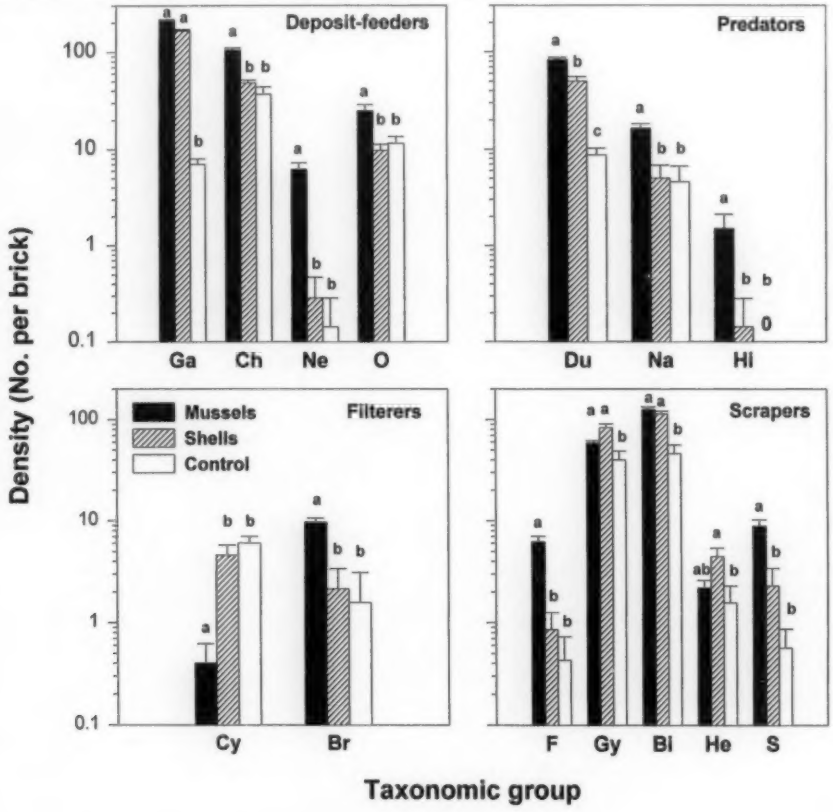
Fig. 5. Mean density (±1 SE) of invertebrates (sorted by functional feeding group) on treatment bricks in the artificial substrate experiment. He, *Helicopsyche*; Gy, *Gyraulus*; Bi, *Birgella*; F, *Ferrissia*; S, *Stenonema*; Ga, *Gammarus*; Ne, *Nectopsyche*; Ch, chironomids; O, ostracods; Cy, *Cyrnellus*; Br, *Brachycentrus*; Du, *Dugesia* (flatworm); Hi, leeches; Na, naidid oligochaetes (primarily *Chaetogaster*).



range of densities at which significant changes were observed (e.g., 1500–4000 mussels/m^2) are likely to occur in most lakes and rivers in North America (Strayer 1991; Ramcharan et al. 1992; Mellina and Rasmussen 1994).

Characteristics of zebra mussel aggregations that influence invertebrate response

Zebra mussels have a suite of physical and biological attributes that may influence the structure of their macroinvertebrate assemblages. Through efficient filtration, zebra mussel populations can remove large amounts of particulate organic matter from the water column and deposit these on sediments as agglutinated mussel feces and pseudofeces (Izvekova and Lvova-Katchanova 1972; Stanczykowska and Planter 1985). Mussel biodeposits are a nutrient-rich and easily assimilated food source (Izvekova and Lvova-Katchanova 1972), and the organic enrichment of substrata by mussel biodeposition can alter the local distribution and abundance of benthic invertebrates (Sephton et al. 1980). Zebra mussel biodeposition is assumed responsible for increased abundances of deposit-feeding chironomids and oligochaetes in Lake St. Clair (Griffiths 1993) and in European lakes (Lyakhnovich et al. 1982; Smit et al. 1993). In our field survey, we found 5- to 10-fold increases in abundances of deposit feeders at the St. Lawrence River sites where dense (>1500 mussels/m^2) zebra mussel populations became established (Fig. 7). However, our artificial substrate experiment suggests that this response was provoked by both physical and biological attributes of zebra mussels.

Mussel beds increase the surface area and spatial heterogeneity of benthic substrata, thereby creating habitat for a diverse group of associated organisms (Suchanek 1979, 1986; Jacobi 1987; Seed 1996). Spatially complex surfaces tend to support richer faunal communities (e.g., Abele 1974; Hart 1978; Minshall 1984; O'Connor 1991), presumably because they provide a greater number of niches and resources. Clumped mussels have an abundance of interstitial spaces that may serve as refugia from disturbance and predation for small organisms (Gosselin and Chia 1995), a role played by other biological substrata (e.g., Brock 1979; Gillinsky 1984; Ban and Nelson 1987; Holmlund et al. 1990; Matsumasa 1994). These spaces also trap sediments and biodeposits (Jacobi 1987; Yager et al. 1993), thus benefiting deposit-feeding organisms. The interaction between interstitial habitat and sedimentation makes it difficult to determine the primary mechanism(s) by which mussel beds enhance populations of associated fauna. Slepnev et al. (1994) found that invertebrate colonization was three times higher in the presence of living

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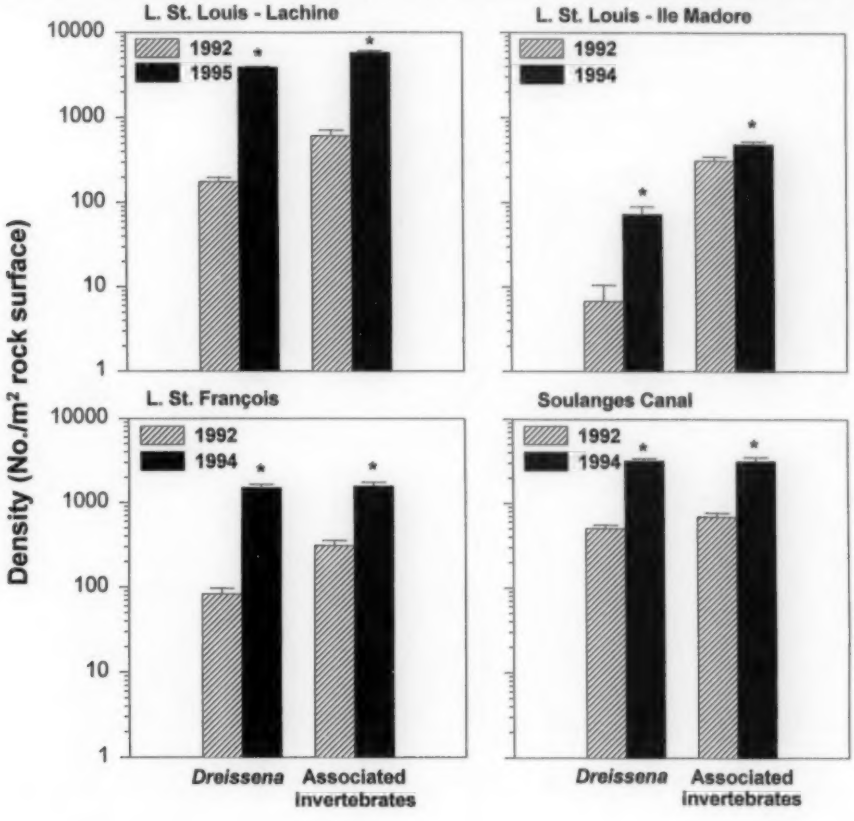
Fig. 6. Mean density (±1 SE) of zebra mussels (*Dreissena*) and associated macroinvertebrates on stones at St. Lawrence River sites. *, $p < 0.005$.



zebra mussels than in the presence of rubber models; they attributed this difference to both the accumulation of biodeposits among living mussels and the more complex spatial structure of natural mussel aggregations. However, Suchanek (1979) showed that artificial mussel beds contained faunal communities virtually identical to those of real *Mytilus* beds. The PCA of data from our artificial substrate experiment (Fig. 3) indicated that although general trends exist, taxa within the same functional feeding group responded differently to treatment, suggesting that they responded to more than just a trophic effect. For several taxa, the effect of mussel structure on abundance was stronger than the various biotic effects associated with living mussels (Figs. 4 and 5). Therefore, although biological attributes of living zebra mussels played significant roles, we believe that the enhancement of substrate complexity by zebra mussel beds was generally the most important factor controlling the changes in macroinvertebrate composition and abundance at our St. Lawrence River sites.

Several previous studies have documented an increased abundance of gammarid amphipods on substrata densely colonized by zebra mussels (Protasov and Afanasyev 1990; Dermott et al. 1993; Griffiths 1993; Stewart and Haynes 1994; Wisenden and Bailey 1995; Botts et al. 1996). Our analysis of field data confirms a general multifold increase in *G. fasciatus* densities coinciding with the development of dense zebra mussel populations in the Great Lakes – St. Lawrence River system. The results of our artificial substrate experiment suggest that this increase is a response to enhanced substrate complexity, which is consistent with published observations that *Gammarus* preferentially colonizes interstitial habitat (Gee 1982; Olyslager and Williams 1993).

Zebra mussels may colonize soft substrata by settling on "seed" surfaces, i.e., individual shells or stones that serve as foci for aggregations of byssally attached mussels (Hunter and Bailey 1992). These aggregations act as islands of firm substrate for epifauna such as hydroids, sponges, bryozoans, turbellarians, gastropods, and leeches, in habitats where hard substrata are scarce (Maslowski 1992; Smit et al. 1995; Botts et al. 1996). However, the dense colonization of mussel-covered bricks by a rich macroinvertebrate community in the presence of abundant bare cobble at our experimental site, coupled with a density enhancement (~400%; Fig. 2) that is disproportionate with the net increase in surface area created by mussel beds (~60%), suggests that zebra mussels provide additional advantages for epibenthic animals.

Filtration currents generated by zebra mussels may be exploited by small organisms such as young sponge colonies and filter-feeding caddisfly larvae (e.g., *Brachycentrus*), which normally attach themselves to the posterior margins of the mussel shell in close proximity to the siphons (A. Ricciardi, personal observation). A similar commensal relationship

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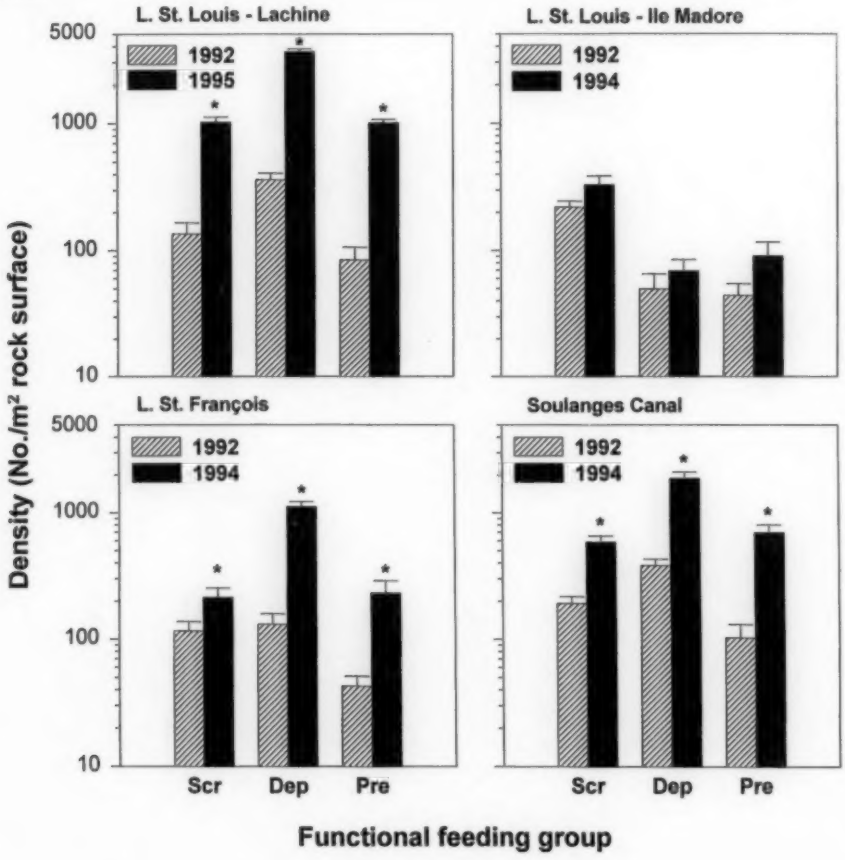
Fig. 7. Mean density (±1 SE) of functional feeding groups on stones at St. Lawrence River sites. Scr, Scrapers; Dep, deposit feeders; Pre, predators. *, $p < 0.05$.



involving blue mussels (*Mytilus edulis*) and barnacles (*Balanus improvisus*) has been documented; barnacles tend to attach to the siphonal regions of mussels, and those that attach to living mussels grow faster than those on empty shells (Laihonen and Furman 1986). *Brachycentrus* larvae orient themselves so that the anterior opening of their case is exposed to the zebra mussel's filtration current; in this position, the larvae appear capable of scavenging incoming food partices. Their preference for living zebra mussels as attachment sites was clearly demonstrated in our artificial substrate experiment (Fig. 5). Furthermore, *Brachycentrus* larvae have densely colonized zebra mussel beds in sections of the St. Lawrence River where they were historically absent (Conn and Conn 1993; D.B. Conn, University of Sewanee, Sewanee, Tenn., personal communication). Sponges are also common epibionts of zebra mussels in the St. Lawrence River (Ricciardi et al. 1995*b*), but their aerial coverage of hard substrata does not appear to be affected by dense zebra mussel colonization (Table 1).

Another organism commensally associated with zebra mussels in our artificial substrate experiment and field studies was the limpet *Ferrissia rivularis* (Ancylidae). Densities of *Ferrissia* in the Soulanges Canal increased fivefold between 1992 and 1994 (Table 1) and were more abundant on zebra mussel shells than on surrounding substrate. Conn and Conn (1993) also found *F. rivularis* occurring in large numbers on zebra mussel beds. *Ferrissia* may benefit from grazing on mussel shell surfaces, which are often covered with attached diatoms (A. Ricciardi, personal observation), the preferred food item for limpets (Thorp and Covich 1991). Because limpets appear to have higher oxygen requirements than most other gastropods (Pennak 1989), they may also benefit from being in close proximity to currents generated by mussel siphons. In our substrate experiment, *Ferrissia* colonized living mussels in larger numbers than either dead mussel shells or bare substrate. By contrast, other scrapers (i.e., *Helicopsyche, Birgella, Gyraulus*) did not differentiate between living and dead mussels, but colonized mussel-covered bricks more densely than bare bricks (Figs. 3 and 5). The depuration of nutrients (N, P) from living and dead zebra mussel shells (Stanczykowska and Planter 1985) could conceivably stimulate periphyton growth and thus attract scrapers.

Zebra mussels may also enhance local invertebrate abundance by acting as prey (e.g., for leeches), or as hosts for parasites and internal commensals. Leeches, particularly glossiphoniids, are common in zebra mussel aggregations (this study; Lewandowski 1976; Griffiths 1993; Dermott et al. 1993; Stewart and Haynes 1994) and may prey on zebra

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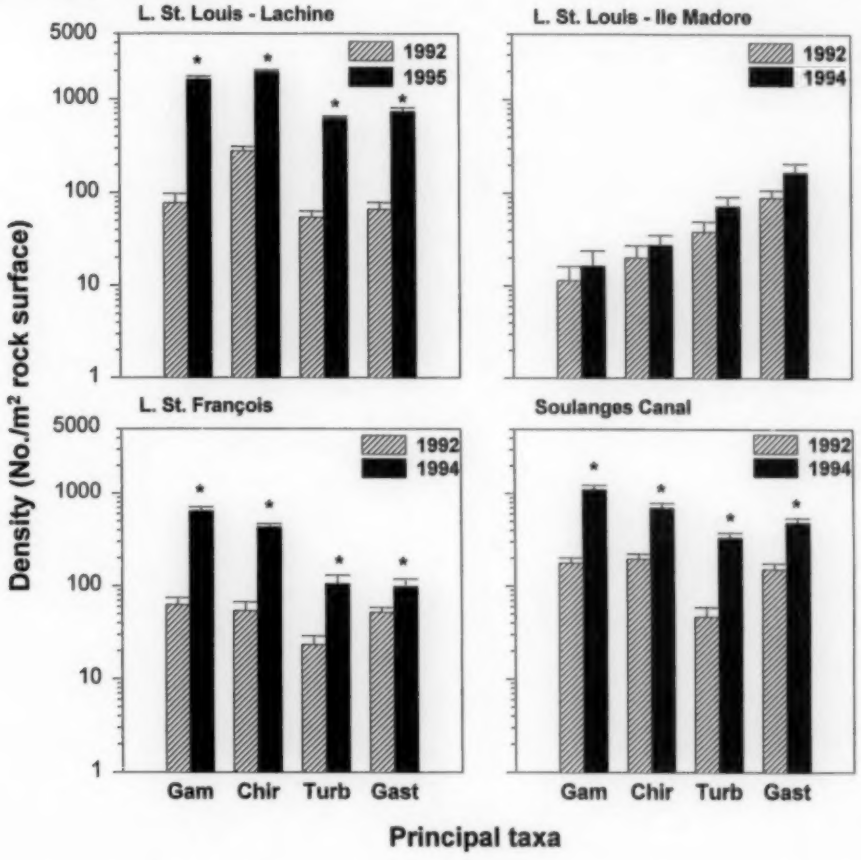
Fig. 8. Mean density (±1 SE) of principal invertebrate taxa at St. Lawrence River sites. Gam, *Gammarus*; Chir, chironomids; Turb, turbellarian flatworms; Gast, snails and limpets. *, $p < 0.05$.



mussels (Smit et al. 1993) or their associated invertebrates (Pennak 1989). The predatory oligochaete *Chaetogaster limnaei* is a facultative parasite of zebra mussels (Conn et al. 1996) and may also be abundant in zebra mussel beds (this study; Piesik 1983; Stewart and Haynes 1994). Larval chironomids of *Paratanytarsus* sp. live commensally in the mantle cavity of zebra mussels, where they are provided with food (in the form of pseudofeces and mucus secretions) and spatial refuge (Ricciardi 1994).

Negative responses to zebra mussels

Contrary to most previous studies (Griffiths 1993; Dermott et al. 1993; Stewart and Haynes 1994), our results suggest that dense coverage of hard surfaces by zebra mussels may reduce or displace certain species. Large net-spinning caddisfly larvae (Polycentropodidae) declined after the establishment of zebra mussel populations at our field sites (Table 1) and avoided substrata covered by living zebra mussels in our artificial substrate experiment (Fig. 5). Competition for food and space limits the distribution and abundance of net-spinning caddisflies on stones (Lancaster et al. 1990; Hemphill 1988). We believe that these larvae cannot exploit zebra mussel filtration currents as can smaller caddisflies (e.g., *Brachycentrus*). Large net-spinning caddisflies are territorial and space themselves to avoid receiving water previously filtered by their neighbors (A. Ricciardi, personal observation; Hemphill 1988). Zebra mussels may compete with polycentropodid caddisflies for optimal positions on substrate, and their filtration activity may produce local flow patterns that interfere with polycentropodid feeding (cf. Johnson 1990).

The decline of the ectoproct bryozoan *Plumatella fungosa* at our Lachine site is unusual given that this species was abundant at this site in previous years (Ricciardi and Reiswig 1994; A. Ricciardi, personal observation) and that plumatellids can use zebra mussels as substrate (e.g., Maslowski 1992). However, because *P. fungosa* builds massive spreading colonies on smooth surfaces in the St. Lawrence River (A. Ricciardi, personal observation), its growth may be limited by the small cavities and discontinuous shell surfaces of clumped mussels. Competition with zebra mussels for local particulates may have also occurred. Conversely, the decline may have been caused by a factor unrelated to zebra mussels, such as a change in water quality (see Ricciardi and Reiswig 1994, and references therein).

The displacement of large snails (i.e., individuals comparable in size with zebra mussels) from substrata densely colonized by zebra mussels has been reported previously (Dusoge 1966; Wisenden and Bailey 1995). Gizinski and Wolnomiejski

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(1982) noted a reduction in native molluscs coinciding with the development of shoals of zebra mussels in a Polish reservoir but did not speculate on mechanisms linking the two events. Although zebra mussels may negatively impact large gastropods by fouling their shells (Tucker 1994), mussels rarely attached to snails at our study sites and probably did not reduce their abundance through increased mortality. Large snails may be inefficient at grazing on interstitial surfaces and thus may have been outcompeted for food and space by smaller gastropods (cf. Harman 1968). We suggest that the alteration of hard substrata by zebra mussel aggregations increases the abundance of snails but limits their mean size, analogous to the effect that marine mussel beds have on gastropods (Jacobi 1987; Griffiths et al. 1992). However, in littoral areas of lakes invaded by zebra mussels, gastropods of all sizes could increase in abundance outside of mussel beds in response to higher benthic algal growth resulting from the enhanced light penetration and nutrient deposition associated with zebra mussel filtration activity (Griffiths 1993; Stewart and Haynes 1994).

Implications for food webs

We expect that higher abundances of *Gammarus*, chironomids, and small gastropods will increase prey availability for benthivorous fishes such as yellow perch (*Perca flavescens*), which feed preferentially on these organisms (Boisclair and Leggett 1989). Examination of the digestive tracts of yellow perch and rock bass (*Ambloplites rupestris*) from the Soulanges Canal at Pointe-des-Cascades, Que., has shown that these fish consumed proportionally more *Gammarus* in the summer of 1995 than in previous years (A. Ricciardi, unpublished data). Moreover, a European study found that the production of benthivorous fish in a lake doubled within 6 years following zebra mussel invasion; this was attributed to both direct predation by fish on zebra mussels and indirect predation on mussel-associated organisms (Karataev and Burlakova 1995). Based on the assumption that mussel biodeposits stimulate *Gammarus* production, Bruner et al. (1994) hypothesized that zebra mussels increase the bioaccumulation of organic contaminants through aquatic food chains (e.g., seston – mussel feces – *Gammarus* – fish – bird). However, their assumption is not supported by the results of our study (Fig. 5) and Botts et al. (1996), both of which suggest that gammarid populations are enhanced by habitat structure rather than mussel biodeposits. It remains to be tested whether gammarids or other deposit feeders that live in zebra mussel beds have higher contaminant concentrations than those living outside of mussel beds.

Conclusions

Our findings indicate that zebra mussel invasions cause rapid transformations of epilithic communities into assemblages dominated by zebra mussels, amphipods, chironomids, flatworms, and small gastropods. Data suggest that larger fauna adapted to smooth substrate (e.g., pleurocerid snails, certain net-spinning caddisflies and bryozoans) may be reduced or eliminated by dense zebra mussel colonization. A number of physical and biological characteristics of mussel beds (e.g., accumulations of biodeposits; filtration currents) may contribute to these changes, but an important factor is the formation of an interstitial shell matrix that provides refuge, traps sediment, and enhances substrate complexity. Multifold increases in the abundance of mussel-associated fauna may occur at zebra mussel densities as low as 1500 individuals/m^2 within a few years of invasion. Given that most lakes and rivers on this continent are physically and chemically suitable for the establishment of these mussel densities (Ramcharan et al. 1992; Mellina and Rasmussen 1994), we predict widespread restructuring of North American freshwater benthic communities and food webs as the zebra mussel continues to invade new drainages.

Acknowledgements

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Competition between stream-dwelling cutthroat trout (*Oncorhynchus clarki*) and coho salmon (*Oncorhynchus kisutch*): effects of relative size and population origin

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Abstract: We conducted competitive trials between stream-dwelling, juvenile cutthroat trout (*Oncorhynchus clarki*) and coho salmon (*Oncorhynchus kisutch*) in laboratory stream channels to examine the effects of relative size and population origin on cutthroat foraging and agonistic behavior. Two experiments were conducted: one-on-one trials (pairs of cutthroat and coho) and serial removal trials (groups of three cutthroat/coho pairs). Each experiment was run using two distinct populations of cutthroat: allopatric cutthroat that had been historically isolated from coho by a barrier falls and sympatric cutthroat that naturally cooccurred with coho. Competitive ability and dominance were indexed by relative (proportional) foraging success and aggression. In the one-on-one trials, allopatric cutthroat were stronger interspecific competitors (versus coho) than sympatric cutthroat, and size-matched cutthroat outperformed size-impaired cutthroat. Within cutthroat/coho pairs, allopatric cutthroat outperformed coho when size matched, but not when size impaired, whereas coho outperformed sympatric cutthroat when given a size advantage, but not when size matched. In serial removal trials, both populations of cutthroat outperformed coho. These results suggest that size is perhaps equally important as species identity in determining competitive dominance between sympatric populations of cutthroat trout and coho salmon.

Résumé : Nous avons effectué, dans des bassins de laboratoire recréant les conditions d'un cours d'eau, des études de la compétition entre la truite fardée (*Oncorhynchus clarki*) et le saumon coho (*Oncorhynchus kisutch*) juvéniles du milieu lotique afin d'examiner les effets de la taille relative et de l'origine de la population sur le comportement alimentaire et agonistique de la truite fardée. Deux expériences ont été effectuées : des essais individuels (paires truite fardée/de saumon coho) et des essais avec retraits consécutifs (groupes de trois paires de truites et de saumons). Chaque expérience a été menée sur deux populations distinctes de truite fardée : des truites fardées allopatriques isolées depuis longtemps des saumons cohos par des chutes formant barrière et des truites fardées sympatriques qui cohabitent dans la nature avec le saumon coho. La capacité compétitive et la dominance ont été établies en fonction du succès relatif (proportionnel) de l'alimentation et de l'agression. Dans les essais individuels, les truites fardées allopatriques se sont révélées des compétiteurs interspécifiques plus puissants (face aux cohos) que les truites fardées sympatriques, et les truites de même taille que les cohos ont affiché des performances supérieures aux truites défavorisées par la taille. Dans les paires truite/saumon, les truites allopatriques avaient de meilleures performances que les cohos, quand les poissons étaient de même taille, mais pas si elles étaient défavorisées par la taille, tandis que les cohos affichaient une performance supérieure aux truites sympatriques lorsqu'ils avaient l'avantage de la taille, mais pas si les poissons étaient de même taille. Dans les essais avec retraits consécutifs, les deux populations de truites ont présenté des performances supérieures aux cohos. Ces résultats semblent indiquer que la taille des poissons est peut-être un élément tout aussi important que l'identité de l'espèce pour déterminer la dominance entre des populations sympatriques de truites fardées et de saumons cohos.
[Traduit par la Rédaction]

Introduction

In many stream-dwelling salmonids, competition for food is mediated through direct, interference competition for space (Larkin 1956; Chapman 1966; Allan 1982; Hearn 1987). This interference competition is manifested by territoriality which, in turn, determines patterns of habitat use among conspecifics (e.g., Dill 1978; Pucket and Dill 1985; Grant and Noakes 1988) and may regulate salmonid population densities (Grant and Kramer 1990). Mechanisms of territorial behavior between conspecifics have been investigated for a large number of salmonids, including brown trout (*Salmo trutta*) (Kalleberg 1958), Atlantic salmon (*Salmo salar*) (Kalleberg 1958; Keenleyside and Yamamoto 1962; Symons 1971; Stradmeyer and Thorpe 1987; Metcalfe et al. 1989), brook trout (*Salvelinus fontinalis*) (McNicol and Noakes 1981, 1984; McNicol et al. 1985; Grant and Noakes 1988; Grant et al. 1989; Grant 1990), rainbow trout and steelhead (*Oncorhynchus mykiss*) (Jenkins 1969; Slanley and Northcote 1974; Abbott and Dill 1989), coho salmon (*Oncorhynchus kisutch*) (Dill 1978; Dill et al.

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1981; Fausch 1984; Pucket and Dill 1985), and Arctic grayling (*Thymallus arcticus*) (Hughes and Dill 1990; Hughes 1992*a*, 1992*b*). However, whereas interspecific aggression between native and introduced salmonids has been implicated in the demise of several native species (e.g., Fausch and White 1981, 1986; Griffith 1988; De Staso and Rahel 1994), the effects of territoriality on the performance of two or more naturally co-occurring salmonid species have not been explicitly examined as in other groups of fishes (i.e., damselfishes) (Ebersole 1977; Thresher 1978).

Territoriality is modified by many factors, including the relative size of the competitors, residency ("first come, first serve"), and prior success in competitive bouts (Abbott et al. 1985; Abbott and Dill 1989). These factors have likewise not been considered in territorial disputes between heterospecifics. Instead, competition between salmonids is discussed in the context of habitat segregation (Nilsson 1967; and reviews by Ross 1986; Hearn 1987). This problem is exemplified by the interactions between cutthroat trout (*Oncorhynchus clarki*) and coho salmon.

In areas of sympatry, coho inhabit mostly pool habitats, with cutthroat in glides and riffles. In streams not supporting coho, cutthroat use all available habitats (Glova 1984, 1987). Laboratory experiments suggest that habitat segregation in sympatry is interactive (sensu Nilsson 1967), with coho excluding cutthroat from preferred pool habitats (Glova 1986). In contrast, habitat segregation may be "selective" (sensu Nilsson 1967), reflecting species-specific preferences for various physical conditions (depths, velocities, etc.). For example, in laboratory stream channels, coho chose pools over riffles and glides with and without cutthroat present (Glova 1986). In the wild, coho seek out velocity refuges whereas steelhead (another salmonid commonly sympatric with cutthroat and coho) choose habitats that maximize overhead cover (Fausch 1993). Coho also show a ontogenetic shift late in the first growing season from glides to deeper, lower velocity pools, reflecting potential morphological constraints in faster water (Fausch 1993). This pattern is even more pronounced as water temperatures drop during the ensuing winter (e.g., Bustard and Narver 1975). Bisson et al. (1988) suggested that body morphologies of coho and steelhead are adapted to pool and riffle habitats, respectively, whereas cutthroat lack any particular habitat specializations. This lack of specialization is, in turn, hypothesized to render cutthroat competitively inferior in the preferred habitats of coho and steelhead, explaining their predominance in glides.

In both interactive and selective models of habitat segregation, dominance of cutthroat by coho is inferred indirectly from patterns of habitat use rather than by the potential mechanisms producing them. Mechanisms of habitat segregation may involve interference competition (e.g., territoriality) operating relatively early in the ontogeny of cutthroat and coho. For example, the adults of both species spawn in pool tail-outs (=glides) and riffles (Pauley et al. 1989; Trotter 1989; Groot and Margolis 1991), suggesting that emergence of both species occurs in the same habitats. The most profitable foraging strategy for coho (Fausch 1984), and perhaps cutthroat as well, is territoriality, which is more effective in glides than in pools (Pucket and Dill 1985). Thus, early behavioral interactions between coho and cutthroat in glide habitats may determine subsequent patterns of habitat use.

The outcome of these behavioral interactions may be largely determined by the relative size of competitors. Coho emerge from spawning gravels earlier and at larger sizes than cutthroat (Laufle et al. 1986; Pauley et al. 1989; Groot and Margolis 1991), resulting in an early season coho size advantage. This size differential early in ontogeny may be a key factor in understanding competitive interactions and patterns of habitat use between coho and cutthroat. However, the outcome of direct competitive bouts between these two species has not previously been addressed.

The first goal of this study was to examine the effect of relative size on the outcome of competition between cutthroat and coho in head-to head competitive bouts in a laboratory arena simulating a glide habitat. Because cutthroat occur naturally both in sympatry with coho and as isolated allopatric populations (usually above large barrier falls, e.g., Northcote and Hartman 1988), competitive abilities of various cutthroat stocks with coho may differ based on evolutionary "experience" with these competitors. Competition with coho may lead to the evolution of improved competitive ability (e.g., Hairston 1983) in sympatric populations of cutthroat. The second goal of this study was to examine potential differences in the innate competitive abilities of cutthroat populations experienced and not experienced with competition with coho. Two populations of cutthroat were used: allopatric cutthroat collected from above a barrier falls preventing contact with coho and sympatric cutthroat taken from a stream where cutthroat and coho naturally co-occurred. Specifically, we hypothesized that (1) coho would outcompete cutthroat only when given a size advantage, and thus, competitive outcomes between cutthroat/coho pairs and within larger groups (three pairs) of these competitors will be largely determined by size and (2) sympatric cutthroat would be better competitors with coho than allopatric cutthroat as a result of selection for improved competitive ability in cutthroat populations sympatric with coho.

Materials and methods

Allopatric cutthroat trout were collected from the South Fork of Fulton Creek (Jefferson County, Washington State, U.S.A.). Fulton Creek originates from two first-order tributaries at an elevation of 1000 m on the east coast of the Olympic Peninsula and drains as a second-order stream 9 river km downstream into the Hood Canal, the western arm of the Puget Sound. A 7-m falls at river km 1.5 prevents upstream passage of anadromous salmon and trout, thereby isolating a land-locked population of cutthroat above this point. The sampling site (~200 m in stream length) was located at river km 5.6 on the North Fork. Sympatric cutthroat and coho were collected from Big Beef Creek (Kitsap County, Washington State, U.S.A.), ~25 km from Fulton. Fish were taken along a transect 200 m in length, 0.5 km from its mouth in the Hood Canal. More detailed descriptions of the collection sites are given in Sabo (1995). All fish were collected by seining or with a Smith–Root model 12 backpack electrofisher and dip nets. Sampling was conducted in a variety of habitat types to avoid any behavioral biases that might result from the use of individuals from single habitat types (Nielsen 1992). Collections were made on five occasions between 12 and 26 August 1994, yielding 68 cutthroat from each of Big Beef and Fulton creeks and 172 coho from Big Beef Creek.

Fish were transferred to the holding and experimental facilities at the University of Washington's Big Beef Creek Research Station immediately after collection. Coho and each population of cutthroat

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Table 1. Experimental treatments and replication.

Experiment	Dates	Treatment		Replications
		Population	Size	
1 × 1: cutthroat/coho pairs	19 Aug.–9 Sept. 1994	Allopatric	Size matched	28
		Allopatric	Size impaired	28
		Sympatric	Size matched	27
		Sympatric	Size impaired	31
Serial removal: three cutthroat/coho pairs	19–30 Sept. 1994	Allopatric	Built-in hierarchy	13
		Sympatric	Built-in hierarchy	12

Note: Two experiments were conducted: one-on-one trials (1 × 1) using single pairs of cutthroat and coho and serial removal trials involving three size-matched cutthroat/coho pairs. There were four treatments in 1 × 1 trials involving cutthroat populations obtained from streams where they were either "sympatric" with coho or "allopatric." Each population was tested in two size scenarios: size matched to the nearest millimetre with coho competitors or cutthroat impaired by a 7-mm size differential. Serial removal trials had two treatments: cutthroat of either allopatric or sympatric origin. In each case, three cutthroat/coho pairs making up a size-based hierarchy (see text for details) were tested together.

were held separately in circular tanks (310 L: 73.5 cm deep and 73.5 cm in diameter) at near equal densities of 70 fish/tank. Tanks were housed in a shaded outdoor shelter, and each was supplied with fresh water of artesian origin (mean $T \pm SD = 10.42 \pm 0.28$°C) and a clockwise current generated by the water source. Fish in each tank were fed a group daily ration of 5 g (frozen weight) of previously frozen bloodworms (Chironomidae), which were taken after no more than 2 days by all individuals. All fish were held for at least 1 week prior to experimentation.

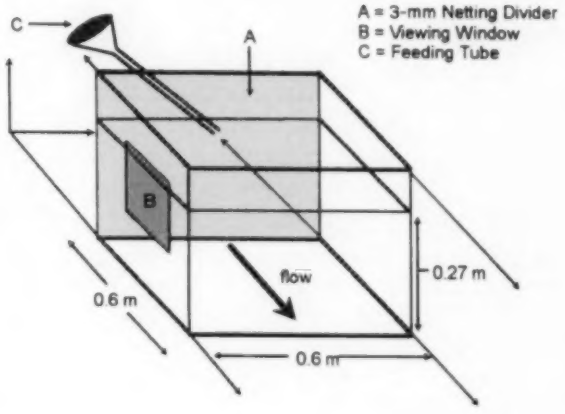
Experiments were conducted in two laboratory stream channels (Fig. 1). These channels were subdivided into 32 experimental units with green polypropylene netting (3-mm mesh). Experimental units were 0.36 m^2, each equipped with a viewing window (45 × 20 cm) allowing for unobstructed view of the entire unit. All units had a substrate of fine gravel 3 cm in depth. An average flow of 4.5 cm/s was maintained using a recirculating pump. Combined, this flow and depth are analogous to glide habitats of small streams of the Pacific Northwest (Bisson et al. 1988). Fresh artesian water (mean $T \pm SD = 10.42 \pm 0.28$°C) was provided at a rate of 20 L/min, resulting a mean channel temperature of 11.75°C (range = 11.37–12.13°C). Ambient temperatures in Big Beef Creek ranged from 15 to 20°C during the experimental period (see Table 1 for dates). No temperatures were recorded for Fulton Creek. Logistic constraints related to the water source and the desire to maintain the same temperature throughtout the entire experimental period (2 months) prohibited finer temperature control. Artificial light (~600 lx) was provided on a 16 h light : 8 h dark photoperiod matching the prevailing August schedule.

Two sets of experiments were carried out: one-on-one (1 × 1) and serial removal trials (Table 1). One-on-one trials involved single cutthroat/coho pairs (4.6 fish/m^2) whereas serial removal trials tested three such pairs en masse (13.8 fish/m^2). Experimental densities were higher than the natural range (0.5–2.0 fish/m^2) reported for coastal Washington streams in August (Bjornn and Reiser 1991). However, densities in one-on-one trials were not greater than those reported naturally in other locations (e.g., 5.6 coho/m^2 in British Columbia; Fausch 1993). Logistic constraints prevented us from using equal densities (i.e., larger area in serial removal trials) in both experiments.

We conducted one-on-one trials with four treatments of cutthroat/coho pairs using a two-way factorial design. Each popuiation of cutthroat, allopatric and sympatric, was either matched to the nearest millimetre in size (fork length) with coho or "impaired" by a standard size differential of 7 ± 1 mm (fork length). The 7-mm size impairment represented the average of the mean size differences between sampled populations of coho and each population of cutthroat. Mean length of coho (62.6 ± 6.4 mm) and both populations of cutthroat (Fulton cutthroat 53.0 ± 7.8 mm, Big Beef cutthroat 57.8 ± 10.0 mm) used in the trials were within 1 SD of means of field-collected populations.

At approximately 15:00 on the day prior to the first day of testing,

Fig. 1. One experimental unit isolated within the larger flume by 3-mm-diameter netting shown here on only one side and supplied with a viewing window and a feeding tube.



16 pairs of test fish were anesthetized in MS 222. The first fish of each pair was chosen randomly from the holding tank and the second matched within 1 mm of the corresponding preassigned treatments (size matched or size impaired). The first fish of each pair was alternately a cutthroat or a coho to avoid nonrandom sampling of the size ranges of each species. Treatments were blocked spatially and temporally (16 units/experimental period, eight experimental periods), but were otherwise assigned randomly to the 16 experimental units. Each fish was allowed to acclimate to the test environment in solitary confinement in one of the 32 total units for at least 20 h, including one solitary feeding (9 bloodworms/fish). Feeding was achieved by serial introductions of individual bloodworms (spaced by 1-min intervals) through the feeding tubes. Fish not eating a majority of the acclimation ration (<5/9) were not used in further testing to remove the potential bias of fish not responding to the experimental protocol.

After solitary acclimation (at approximately 14:00 on the first day subsequent to introduction to the channels), pairs were transferred to experimental units following the randomized block design. Testing began the following morning at 08:00 consisting of six feeding rounds (three bloodworms each spaced by 1-min intervals) for a total of 18 bloodworms/pair. Each feeding round was spaced by 45 min, resulting in a 3.75-h trial period. The number of bloodworms acquired by each fish was recorded. Further, aggressive encounters were classed as nips, chases, or displays and tallied for 1 min following each feeding round.

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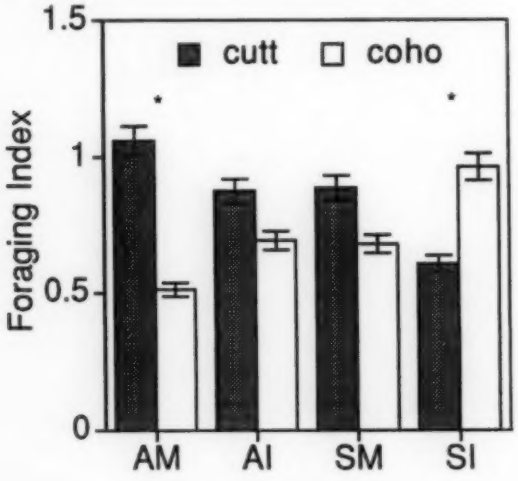
Fig. 2. Mean foraging success (±SE) for size-matched (M) and size-impaired (I) sympatric (S) and allopatric (A) cutthroat and their coho partners. The foraging index represents arcsine square-root transformed mean proportional foraging success (no. captured/total no. presented, range = 0.12–1.45). Asterisks indicate significant differences between cutthroat/coho pairs ($P < 0.05$; $P = 0.053$ in the size-impaired sympatric treatment; paired t-tests).



Proportions of foraging success (n/18) for each fish were arcsine square-root transformed after adjusting for extreme values ($0/n = 1/4n$, $n/n = 1-1/4n$; Zar 1984). A randomized block ANOVA was then performed, with the transformed values as the dependent variable, treatment type ($k = 4$) as the independent variable, and test period ($k = 8$) and longitudinal position ($k = 4$) as two separate blocks. When no block effects were found ($F = 4.48$ and 1.28, $P = 0.29$ and 0.65, df = 3 and 3 for time and space blocks, respectively), the transformed data were analyzed in two ways. First, a two-way ANOVA was used to evaluate differences in cutthroat performance among the four treatments. Differences between cutthroat and their coho partners in each of the four treatments were then analyzed with paired-samples t-tests. Analyses of aggressive behavior were performed on four categories of aggression data: nips, chases, displays, and pooled aggression. Differences among treatments (cutthroat) were analyzed using a Kruskal–Wallis test using the experimental treatments as four separate cases in a one-way design. Differences between cutthroat and coho were analyzed with Wilcoxon signed rank tests.

Serial removal trials differed from one-on-one trials in both the number of fish used in each trial (six versus two) and the experimental protocol used to identify competitive effects on foraging success. Two treatments, allopatric (Fulton cutthroat) and sympatric (Big Beef cutthroat), were used involving three cutthroat/coho pairs each. Each pair was matched in size to the nearest millimetre, and each successive pair in a group differed by 7 ± 1 mm. At 15:00 on the day prior to the experimental period, fish were anesthetized in MS 222 and measured to the nearest millimetre. All fish had been used previously in the one-on-one treatments, and thus were not acclimated individually to the test environment. Fish were reused to minimize collection of wild fish. To minimize the effect of prior success in one-on-one bouts on competitive status in the serial removal trials, all fish were held together in the holding tanks for at least 10 days prior to conducting the latter trials.

Six fish were added to each experimental unit following measurement and allowed to acclimate together for ~40 h. At 08:00, feeding trials were initiated. On each testing day, fish were fed bloodworms using a procedure similar to that in one-on-one trials. Because more fish were used in serial removal trials, the daily test ration was increased to 24 (four groups of six) for the serial removal trials. Thus, six bloodworms were introduced at 1-min intervals during each of four trial feedings spaced by ~1.5 h (total trial time = 4.5 h). The number of bloodworms acquired was recorded. Aggression was also recorded as above. However, due to the large number of combinations of aggressive encounters involved with six test fish, an index of aggression based on the number of encounters per individual towards all other fish was derived.

The testing procedure, adapted from Metcalfe et al. (1989), involves successive daily removals of the most dominant fish based on the number of prey consumed. A "dominant" fish acquired at least twice as many bloodworms as the individual with the second highest food score within each replicate. Winning scores ranged from 8–4 to 24–0. A tie was declared when the top score was less than twice that of the second highest score. In these cases, a "winner" was chosen based on aggression scores. In all cases, daily dominants, or winners, were removed from the experiment. Dominants were assigned a rank corresponding to the number of fish left in the test when removed (none to five). In the case of ties, both fish received an average of their two ranks. This procedure was repeated each day for 5 days, until one fish remained. Average ranks for coho and cutthroat were generated for each replicate, yielding a series of cutthroat and coho scores for each treatment ($n = 13$ and 12 for allopatric and sympatric treatments, respectively). Differences between races of cutthroat in group performance were analyzed using t-tests. Paired t-tests were carried out between coho and cutthroat within each treatment to evaluate differences between cutthroat/coho group performance in both cases. All statistical analyses were performed using SPSS version 6.1.

Results

One-on-one trials: foraging success

Both relative size and cutthroat population origin had significant effects on cutthroat foraging success (size: $P = 0.01$, population: $P = 0.014$; ANOVA), but there was no interaction between these two factors ($P = 0.58$). In general, cutthroat size matched with coho had greater foraging success than cutthroat impaired by a standard size differential, and allopatric cutthroat had higher foraging success than their sympatric counterparts (Fig. 2). Allopatric cutthroat significantly outperformed coho when size matched ($P = 0.00\,1$; paired t-tests), but not when 7 mm smaller, or size impaired ($P = 0.284$). In contrast, sympatric cutthroat performance was lower than that of allopatric cutthroat in both size pairings with coho. Sympatric cutthroat size matched with coho had higher mean foraging success, but this difference was not significant ($P = 0.32$), whereas size-impaired sympatric cutthroat had lower foraging success than their coho partners ($P = 0.053$). Hence, allopatric cutthroat "win" against equally sized coho, and sympatric cutthroat "lose" to larger coho, whereas size-impaired allopatric and size-matched sympatric cutthroat are more or less evenly matched with coho competitors.

Aggression

Aggression data were variable, ranging from 0–0 ties and close, low-scoring bouts (3–2) to clear dominance (>30–0) of one fish over the other. There were no significant differences among cutthroat in the four treatments for the four categories of aggression: nips ($P = 0.53$; Kruskal–Wallis tests), chases ($P = 0.19$), displays ($P = 0.75$), or pooled aggression ($P = 0.72$). However, there were several differences between

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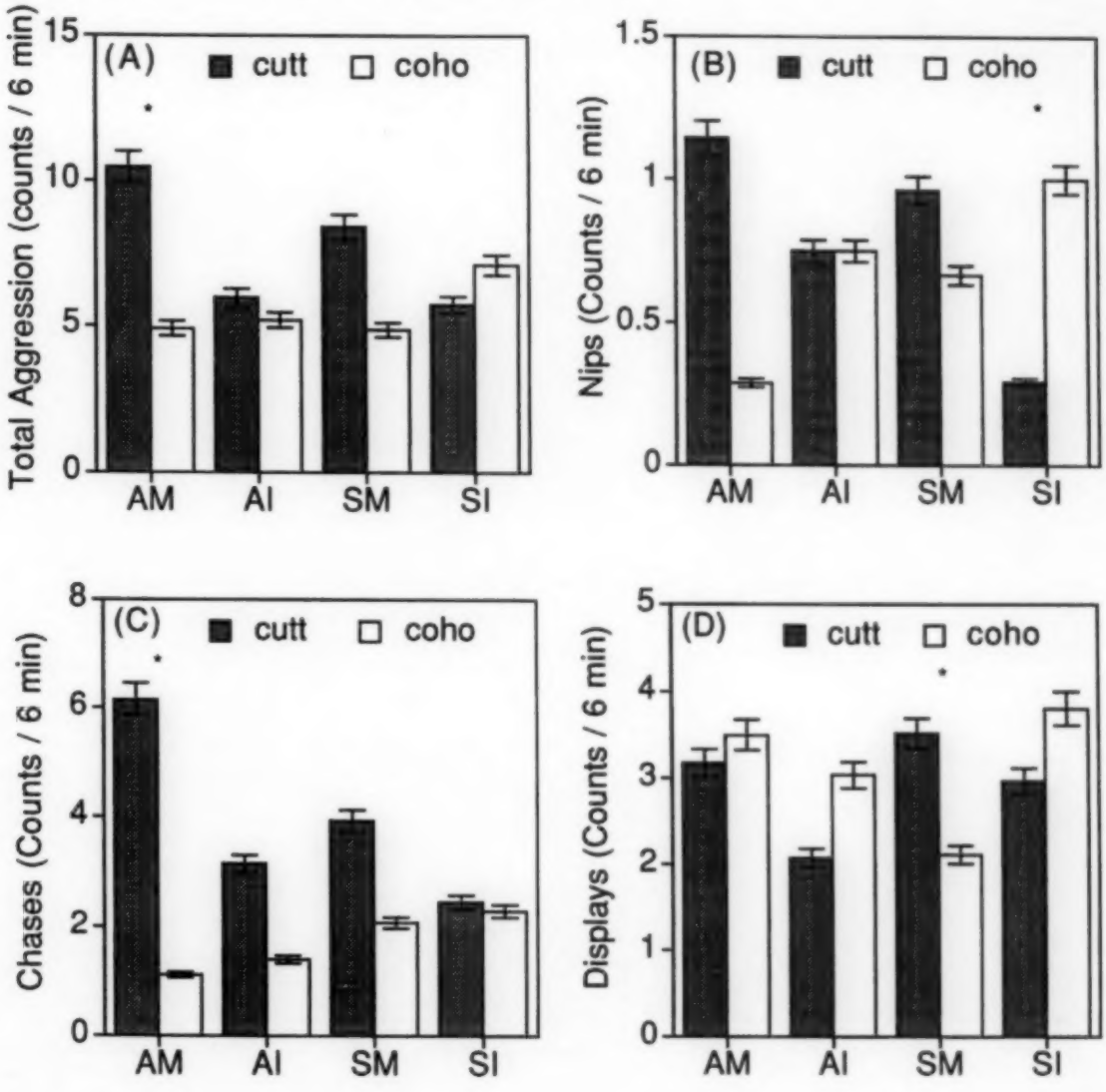
Fig. 3. Mean counts (±SE) of (A) pooled aggression (= nips + chases + displays, (B) nips, (C) chases, and (D) displays for size-matched (M) and size-impaired (I) allopatric (A) and sympatric (S) cutthroat and their coho partners (for six 1-min periods following feeding rounds). An asterisk indicates a significant difference ($P < 0.05$; Wilcoxon's signed rank test).



cutthroat and coho within each treatment for several of these comparisons (Fig. 3).

For example, allopatric size-matched cutthroat exhibited higher mean pooled aggression and chased their counterparts more than coho ($P = 0.03$ and 0.01, respectively, Wilcoxon signed rank tests). Sympatric size-matched cutthroat exhibited higher mean pooled aggression than coho that was nearly significant ($P = 0.08$) and displayed significantly more frequently than their coho counterparts ($P = 0.05$). Thus, when comparing size-matched treatments, allopatric cutthroat were more aggressive than coho by using chases whereas sympatric cutthroat accomplished this via displays.

In contrast with high mean aggression of size-matched cutthroat relative to coho, size-impaired cutthroat (both races) exhibited weaker performance in all categories of agonistic behavior. These results were all nonsignificant except in the sympatric, size-impaired treatment where nips by coho were significantly greater than those by cutthroat ($P = 0.03$).

Serial removal trials

In serial removal trials, cutthroat performance was enhanced for both races (Fig. 4). However, there were no significant differences in overall cutthroat rank between sympatric and allopatric treatments when all size-classes were pooled ($P = 0.416$, $n = 36$ and 39 for sympatric and allopatric treatments, respectively). Both cutthroat races significantly outperformed

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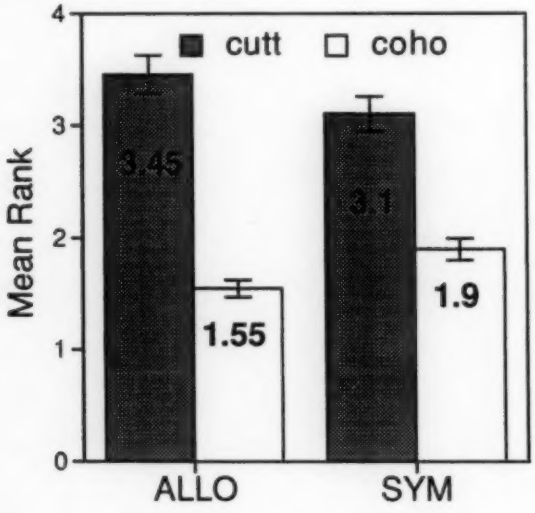
Fig. 4. Mean rank (±SE), all three size-classes pooled and averaged, for allopatric (ALLO) and sympatric (SYM) cutthroat and their coho partners in the serial removal trials. Rank is based on the number of fish left in the trial on the day of removal (e.g., high score of 5 = fish removed on day 1 leaving behind five fish). Differences between cutthroat and coho in both treatments are significant ($P < 0.05$; paired t-tests).



their coho counterparts as reflected by mean rank ($P < 0.0001$ and < 0.0001, $n = 36$ and 39 for sympatric and allopatric treatments, respectively). Thus, performance of sympatric cutthroat was better in serial removal than in one-on-one trials, whereas that of allopatric cutthroat did not change.

Discussion

The goal of this study was twofold: (1) to assess the effect of relative size on the outcome of head-to-head competitive bouts between juvenile coho and cutthroat and (2) to investigate the evolutionary consequences of competition between cutthroat and coho on cutthroat foraging and agonistic behavior. To reiterate predictions made earlier, we postulated that coho would outcompete cutthroat when given a size advantage over these competitors. We also hypothesized that sympatric cutthroat, having historical competitive experience with coho, had evolved improved competitive ability when compared with naive, allopatric cutthroat. In general, the results of our experiments suggest the following: (*i*) coho outcompete sympatric cutthroat only when given a size advantage (prediction 1 is partially supported) and (*ii*) allopatric cutthroat are better competitors with coho than sympatric cutthroat, outcompeting coho when matched in size with these competitors (prediction 2 is rejected).

Competition between cutthroat and coho reassessed

Since Chapman's (1966) assertion that food limitation in stream-dwelling, juvenile salmonids was mediated through space limitation (e.g., territoriality), habitat use has been used almost exclusively as the indicator for competitive dominance among these fishes (e.g., Hearn 1987). Following Chapman's (1966) hypothesis, habitat overlap has been assessed for nearly every two-way combination of stream-dwelling salmonid possible, including chinook (*Oncorhynchus tshawytscha*) and coho (Lister and Genoe 1970; Stein et al. 1972; Taylor 1988, 1991), chinook and steelhead (Edmunson et al. 1968; Everest and Chapman 1972), coho and steelhead (Hartman 1965; Fraser 1968), steelhead and cutthroat (Hartman and Gill 1968), and coho and cutthroat (Bustard and Narver 1975; Glova 1984, 1986, 1987; Bisson et al. 1988). However, in nearly all of these studies, one of the paired experimental or observed fish is declared competitively superior based on its dominance of a "preferred" habitat (but see Fraser 1968; Kennedy and Strange 1980). Following this logic, coho have been declared competitively superior to cutthroat as a result of the demonstration of interactive segregation between the two species (Glova 1984, 1986, 1987). Our results from head-to-head competitive bouts between these two species, using foraging success and interspecific aggression as indices of competitive ability, suggest two possible amendments to the notion that coho are more aggressive and competitively dominant than cutthroat. First, the outcome of competition between single pairs of coho and cutthroat (especially sympatric cutthroat) is largely dependent on the size differential between the two fish. Second, the performance of cutthroat is improved when tested in groups.

Size-matched cutthroat are stronger competitors against coho than cutthroat impaired by a 7-mm size disadvantage regardless of population origin. Further, whereas size-impaired sympatric cutthroat were narrowly defeated by coho, size-matched cutthroat were on average equal competitors with coho. Hence, only sympatric cutthroat at a considerable size disadvantage (~12–13% fork length) were outcompeted by coho. In most wild sympatric populations of cutthroat and coho, coho emerge from spawning gravels as much as a month earlier, and at a size ~5 mm greater than that of cutthroat (Laufle et al. 1986; Pauley et al. 1989; Trotter 1989; Groot and Margolis 1991). This initial size advantage, augmented by early coho emergence and subsequent growth, may have considerable competitive ramifications. However, the size difference may be attenuated by three factors: (1) a late-season peak in cutthroat growth rate (Bilby et al. 1996), (2) a reduction in the proportional size difference as a result of increased overall size in both fishes, and (3) the longer juvenile freshwater residence of cutthroat (2–4 years longer) which provides the overlap of several age-classes of this species with generally only one age-class of coho (Laufle et al. 1986; Pauley et al. 1989; Trotter 1989). Thus, size-impaired trials may mimic early season (May–June) interactions and size-matched trials later season (August–September) interactions. In any case, the outcome of competition between coho and sympatric cutthroat is less a function of species than of the size differential between the two competitors.

In juvenile steelhead, dominance is likewise determined by size and maintained by ensuing competitive experience (Abbott et al. 1985). The maintenance of the dominance relationship may be enhanced by greater access to food and (or) faster growth rates in the dominant fish which exacerbate the size differential between the two fish (Metcalfe 1986; Abbott and Dill 1989; but see Huntingford et al. 1989). Our results from bouts between sympatric cutthroat and coho suggest that size may also be a key mechanism leading to dominance among heterospecifics.

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In serial removal trials, cutthroat performance was boosted by either the higher number of interactors in these trials or the increased density. Our experiments cannot differentiate between these two alternatives. However, several behavioral observations made during the trials qualitatively support the former. For example, in most cases the fish established aggressive "pecking orders" loosely resembling dominance hierarchies based on size and species (e.g., Hughes 1992*a*, 1992*b*; Fausch 1984; Fausch and White 1986). In these hierarchies, the dominant fish received challenges (nips, chases, and displays) from the next subordinate fish in the hierarchy, and rarely from other subdominants. This fish, in turn, received reprisal from the dominant as well as challenges from at least the next subordinate fish, and often from several other subdominants. With more than two fish present (six in our experiments), the subordinate member of the top-ranking pair of fish must both ward off other sub-dominants and attempt to challenge the fish at the top of the hierarchy. In this way, the hierarchical dominance of fish in larger groups provides more foraging time for the dominant. In our experiments, sympatric cutthroat at the top of the hierarchy (in the largest cutthroat/coho pair) may have benefitted from this increased foraging time leading to a clearer victory over similar-sized coho rather than the stalemates observed in one-on-one trials.

In total, the results from our experiments demonstrate that at least in laboratory conditions analogous to glide habitats, cutthroat are not subdominant to coho in head-to head competitive bouts. These results are surprising in light of previous assessments of competitive interactions between cutthroat and coho based on habitat use (Bustard and Narver 1975; Glova 1984, 1986, 1987). Two other independent lines of evidence dispute the coho dominance conclusion inferred from patterns of habitat use. First, the defense of a feeding territory is the optimal foraging strategy for coho early in ontogeny, a strategy accomplished primarily in glide habitats (Pucket and Dill 1985). This implies that pools are not the preferred early ontogenetic habitat of coho, but possibly secondary habitats to which subdominants are displaced. However, species-specific adaptations to flow may provide an alternative means of predicting interspecific competitive status in specific habitats. For example, coho seek velocity refugia in faster riffle and glide habitats (Fausch 1993) and move to deeper, slower pools in response to higher flow and cooler water temperatures in the winter (Bustard and Narver 1975). Innate habitat preferences may also help to determine habitat distributions (at least of coho) and competitive status. Our experiments did not assess competitive status between cutthroat and coho in simulated pool or riffle habitats, so full extrapolation of our results to a field scenario is not yet possible.

Second, cutthroat may be habitat generalists (Bisson et al. 1988). In Holland Creek, British Columbia, one of the two sympatric streams used in the field surveys generating the interactive segregation conclusion (Glova 1984), cutthroat were evenly distributed between habitats despite the preponderance of coho in pools. Our data, combined with these observations, fail to rule out the alternative possibilities that cutthroat are either unaffected by coho habitat preferences or that they influence coho habitat distributions.

Evolutionary consequences of competition

Allopatric cutthroat had evolved only in the presence of competition with conspecifics, yet were better competitors against coho than sympatric cutthroat. This result suggests that sympatric cutthroat have not evolved improved competitive ability with coho when compared with allopatric cutthroat. We hypothesize that the intensity of intraspecific competition may have been high enough in the allopatric population to promote selection for stronger interference mechanisms. This has been demonstrated elsewhere for plethodontid salamanders (Nishikawa 1987) but remains to be tested in stream-dwelling salmonids.

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Prey selection by juvenile walleye as influenced by prey morphology and behavior

Lisa M. Einfalt and David H. Wahl

Abstract: Mechanisms influencing selection of prey by juvenile walleye (*Stizostedion vitreum*; 100–210 mm) were examined in a series of laboratory experiments. Size preference was determined in aquaria (72 L) by introducing four to six individuals (5-mm length increments) of either bluegill (*Lepomis macrochirus*), gizzard shad (*Dorosoma cepedianum*), or golden shiner (*Notemigonus crysoleucas*) with a walleye from one of five length classes. Based on prey length, walleye selected smaller bluegill (20–32% of walleye total length) than either gizzard shad (24–33%) or golden shiner (38–43%). However, body depths of selected prey were similar across species (5–9% of walleye length). Preferences did not always agree with predictions from an optimal foraging model (handling time/prey dry mass), with walleye choosing larger prey based on both length and body depth. Species preference experiments in 2-m pools showed number of prey captures were higher for gizzard shad (76%) than for golden shiner (17%) and bluegill (7%). To explain differential prey vulnerability, observations of predator and prey behavior were conducted in a 750-L tank. Mean captures per strike were highest for gizzard shad (0.41), followed by golden shiner (0.32) and bluegill (0.13). Behavior and morphology, unique to each prey species, influenced walleye predation success.

Résumé : Nous avons étudié les mécanismes qui influent sur la sélection des proies par les juvéniles de doré jaune (*Stizostedion vitreum*; 100–200 mm) dans des expériences menées en laboratoire. Nous avons déterminé la préférence de taille dans des aquariums (72 L) en introduisant quatre à six spécimens (plages d'augmentation de taille de 5 mm) de crapet arlequin (*Lepomis macrochirus*), d'alose à gésier (*Dorosoma cepedianum*) ou de chatte de l'est (*Notemigonus crysoleucas*) en présence d'un doré jaune appartenant à l'une des cinq classes de longueur établies. D'après la longueur des proies, les dorés choisissaient les crapets arlequins de plus petite taille (20–32 % de la longueur totale du doré) que les aloses à gésier (24–33 %) ou les chattes de l'est (38–43 %). Toutefois, la hauteur du corps des proies choisies était similaire d'une espèce à l'autre (5–9 % de la longueur du doré). Les préférences ne concordaient pas toujours avec les prédictions fournies par un modèle d'alimentation optimale (temps de manipulation/masse sèche des proies), car le doré choisissait des proies plus grandes, sur le plan tant de la longueur que de la hauteur du corps. Des expériences sur les préférences spécifiques dans des bassins de 2 m ont révélé que le nombre de proies capturées était plus élevé pour l'alose à gésier (76 %) que pour la chatte de l'est (17 %) et le crapet arlequin (7 %). Pour expliquer la vulnérabilité différentielle des proies, nous avons effectué des observations du comportement des prédateurs et des proies dans un réservoir de 750 L. Le nombre moyen de captures par attaque était au maximum pour l'alose à gésier (0,41), puis pour la chatte de l'est (0,32) et enfin pour le crapet arlequin (0,13). Le comportement et la morphologie, qui sont particuliers pour chaque espèce de proie, avaient une influence sur le succès de prédation du doré jaune.
[Traduit par la Rédaction]

Introduction

Walleye (*Stizostedion vitreum*) are top predators that show highly variable growth and survival in many aquatic ecosystems. These differences are often attributed to a variable forage base, suggesting that use of some prey sizes and species over others may be a critical component. Although several laboratory studies have examined species preference and prey size for walleye fry (Mathias and Li 1982; Raisanen and Applegate 1983; Mayer and Wahl 1997), few have done so for juveniles (age 0 and 1, 50–250 mm). Difficulties maintaining juvenile walleye in the laboratory have resulted in low sample sizes, evaluation of only a small number of potential factors influencing foraging, and no direct observations of feeding behaviors (Buynak et al. 1982; Lyons 1987). Foraging behavior of other piscivores, such as esocids (Gillen et al. 1981; Moody et al. 1983; Wahl and Stein 1988) and largemouth bass (*Micropterus salmoides*) (Savino and Stein 1982, 1989; Howick and O'Brien 1983; Hambright 1991), have been examined extensively, but walleye differ both behaviorally and morphologically from these predators. Walleye actively feed at lower light intensities and strongly associate with the bottom, utilizing structure for cover (Ryder 1977; Wahl 1995). Because these behaviors may lead to different foraging practices, generalizations from these studies may not be applicable to understanding walleye foraging behavior.

Field studies have indicated that juvenile walleye are size-selective predators (Nielsen 1980; Knight et al. 1984), but

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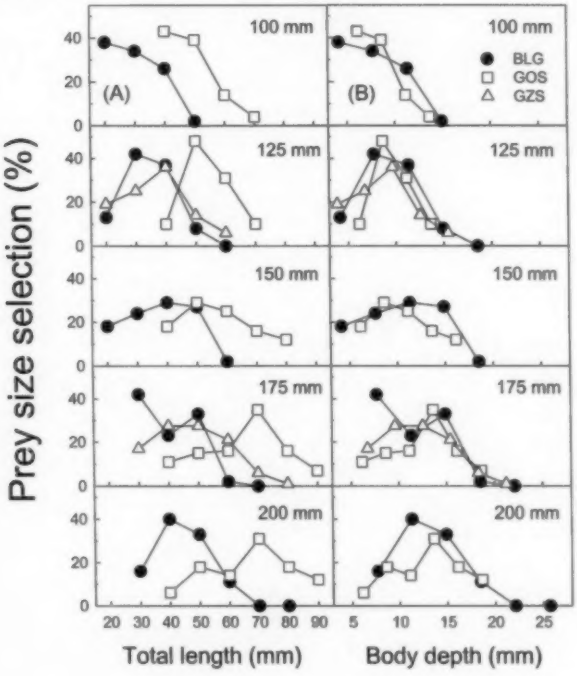
Fig. 1. Proportion of each prey size-class of bluegill (BLG), golden shiner (GOS), or gizzard shad (GZS) selected by different sizes of walleye (100–210 mm) during aquaria experiments. Size-classes chosen are presented for both prey total length (A) and body depth (B). Single-species groups of prey with individuals from each of four to six length groups were fed to each walleye size-class. For each prey and predator combination, at least 45 experiments were completed. For gizzard shad, experiments were only completed for the 125- and 175-mm walleye size-classes.



optimal prey sizes are not known. In addition, soft-rayed species are preferred over spiny-rayed species (Parsons 1971; Knight et al. 1984), but prey density may also influence choice (Lyons 1987). Preference for one prey type over another may affect growth. Lower growth of walleye as been found in centrarchid-dominated lakes as compared with lakes with clupeid or cyprinid forage (Beyerle 1978; Santucci and Wahl 1993), indicating that some prey species may be more vulnerable than others. However, it is difficult to make comparisons among lakes because of the influence of other factors such as lake size, prey species and densities, percent littoral zone, and predator composition (Santucci and Wahl 1993).

It is often assumed that predators actively choose their prey. This concept is central to optimal foraging models, which are often utilized to account for prey sizes and species constituting predator diets (Werner 1974; Gillen et al. 1981; Werner and Mittelbach 1981). To increase fitness, predators choose prey that minimize energy spent on capture and handling while maximizing caloric intake (Werner and Mittelbach 1981). Although this approach adequately explains planktivore foraging, it has been criticized as unrealistic for piscivores foraging on mobile prey. These prey require measurable energy demands involved with stalking and capturing that can vary considerably with size of prey (Sih and Moore 1990). In a review of laboratory studies with controlled prey densities, a variety of predators chose the smallest prey available, and many of these prey were smaller than optimal (Juanes 1994). These results have led to the suggestion that predators are not actively choosing prey but that prey capture is a passive process (Sih and Moore 1990). Under this scenario, predators attack all sizes of prey as encountered, but differential vulnerabilities lead to smaller, more vulnerable prey being consumed in higher proportions (Juanes 1994; Juanes and Conover 1994).

Other studies have focused on prey morphology and behavior as factors determining species selection (Wahl and Stein 1988). Fusiform or soft-rayed prey are generally preferred over centrarchids, and several mechanisms have been proposed to account for these preferences. Prey differ behaviorally in response thresholds (Webb 1986) and escape tactics (Moody et al. 1983; Wahl and Stein 1988; Savino and Stein 1989), and body morphology can influence capture success and handling time (Moody et al. 1983; Webb 1986; Hoyle and Keast 1987; Wahl and Stein 1988; Hambright 1991).

In this study, we investigate size and species preference of juvenile walleye. We chose three common prey species that co-occur with walleye in lakes and reservoirs (Knight et al. 1984; Santucci and Wahl 1993). Bluegill (*Lepomis macrochirus*) and gizzard shad (*Dorosoma cepedianum*) are both deep bodied, but bluegill have dorsal and anal spines, whereas gizzard shad are soft rayed. Golden shiner (*Notemigonus crysoleucas*) are also soft rayed but have a fusiform body shape. Comparisons among these species and sizes allowed us to evaluate the influence of prey morphology and behavior on selectivity.

Methods

Walleye for all experiments were obtained as fingerlings (80–125 mm total length) from the Jake Wolf Memorial Fish Hatchery in Manito, Ill. Fish were acclimated to the laboratory for at least 1 month prior to experiments, and maintained on a diet of fathead minnow (*Pimephales promelas*). Walleye were fed test prey at least 2 weeks before experiments. Bluegill and gizzard shad were seined from local ponds and reservoirs, and golden shiner were obtained from bait dealers. For all experiments, water temperatures ranged from 18 to 21°C and light levels from 150 to 200 lx.

Size-selection experiments

Prey size preference by walleye of several sizes feeding on either bluegill, golden shiner, or gizzard shad was determined in aquaria (72 L) experiments. A single walleye from a 10-mm length class (100–110, 125–135, 150–160, 175–185, and 200–210 mm) was combined with four to six prey, one each from a distinct size-class (5-mm length increments; 20–24, 30–34, 40–44, 50–54, 60–64, 70–74, 80–84, and 90–94). For each walleye length class, we used sizes of prey capable of being eaten by the walleye (see Fig. 1), but the range included at least one prey large enough to determine upper limits of ingestible size, as estimated from preliminary experiments. Hereafter, we refer to predator and prey size-classes by the smallest value in each range. The first prey size captured was recorded, and a capture efficiency for each size-class of prey was calculated as the number of captures divided by the number of strikes. Location of prey capture was recorded as head, midbody, or caudal. Experiments were terminated if no prey were captured within 15 min, but in the majority of experiments, walleye captured prey within 5 min. We completed at least 45 experiments with 9–15 walleye (≤5 trials/individual) of each predator length class and prey species combination. Because of

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seasonal availability of age-0 gizzard shad, only the 125- and 175-mm walleye length groups were tested.

Optimal prey sizes were determined by developing cost–benefit relationships as the ratio of handling time per prey dry mass (Werner 1974). The minimal cost–benefit ratio for each prey and walleye size-class represents the optimal-sized prey (Werner 1974; Gillen et al. 1981). We recorded 10 handling times (time from capture to complete ingestion of prey; Gillen et al. 1981) for each prey and predator length class ($N = 10$ walleye/length class). For all three prey species, total length (nearest 1 mm), body depth (nearest 0.1 mm), and dry mass (nearest 0.001 g) for individuals ($N = 10$) from each prey class were determined; from these data, regression equations were formulated to predict body depth and dry mass from total length.

Species preference experiments

Species preference by juvenile walleye was examined in a 2.5 m diameter pool with white interior walls. Individual walleye (185–210 mm) were held in an opaque plexiglass compartment located along the side of the pool. Bluegill, golden shiner, and gizzard shad ($N = 5$ each) of optimal size (minimal cost–benefit ratio determined from size-selection experiments) were acclimated to the pool 24 h prior to experiments. Ten minutes before releasing a predator, we recorded prey behaviors at 2-min intervals as either schooled (three or more individuals swimming as a unit) or dispersed. Walleye foraged for 24 h, after which the number of each prey species captured was recorded. We used 5 walleye in 8–11 replicates for a total of 51 experiments.

We further examined prey species preference by walleye in paired choice experiments. Two optimally sized prey, one for each of two species, were placed simultaneously into the center of an aquarium (72 L) containing an individual walleye (100–210 mm). We recorded the first prey species captured; experiments were terminated if walleye did not eat within 10 min (3% of all experiments). All possible two-species combinations were tested. For each combination, 15 different walleye were used in 2–5 replicates for a total of at least 50 experiments.

Prey vulnerability experiments

Mechanisms determining vulnerability to predation were examined in a 750-L rectangular glass tank (180 × 70 × 60 cm). A white plexiglass divider separated the experimental tank into two predator holding compartments (30 × 35 cm each) and an experimental chamber (150 × 70 cm). Single species groups ($N = 15$) of naive, optimally sized individuals of either bluegill, golden shiner, or gizzard shad were acclimated for 24 h in the experimental chamber. Before an experiment, walleye (185–210 mm) were starved for 24 h in the holding compartments. An experiment began by releasing a single walleye through a remotely controlled door into the chamber containing prey. During 30-min experiments, predator behaviors were coded into a Datamyte event recorder (Electro/General Corp., Minnetonka, Minn.), which recorded both number of occurrences and time spent in each behavior (0.01 s). Walleye behaviors were separated into seven categories, similar to those defined previously for other piscivores (Savino and Stein 1982; Wahl and Stein 1988): inactive (resting and motionless, not oriented toward prey); observe (motionless, but oriented to an individual prey); search (moving, not orienting to prey); follow (moving and orienting to prey); pursue (following at burst speed); attack (striking at prey); and capture (grasping prey).

We recorded whether prey were schooled (three or more individuals moving as a unit) or dispersed during a strike or capture, and the distance prey were followed or pursued (cm) was estimated from 5-cm marks on the tank walls. For captured prey, the area of the body initially grasped by the predator was determined as head, midbody, or caudal, and handling times were recorded. We used five walleye in four to seven replicates for each prey species to generate 25 experiments for each predator and prey treatment. To avoid problems associated with pseudoreplication (Hurlbert 1984), means of behaviors were compared for individual walleye ($N = 5$ per prey species). Unless indicated otherwise, differences among prey for predator behaviors were assessed with analysis of variance (ANOVA), Tukey's multiple comparisons (Tukey's HSD), and least-squares (LS) means.

Prey behaviors, recorded every 2 min ($N = 15$ observations per experiment), included number schooled and dispersed and the distance (cm) from the predator to the closest schooled or dispersed prey. We tested behavioral differences among prey species with ANOVA, Tukey's HSD, and LS means ($\alpha = 0.05$; SAS Institute Inc. 1988).

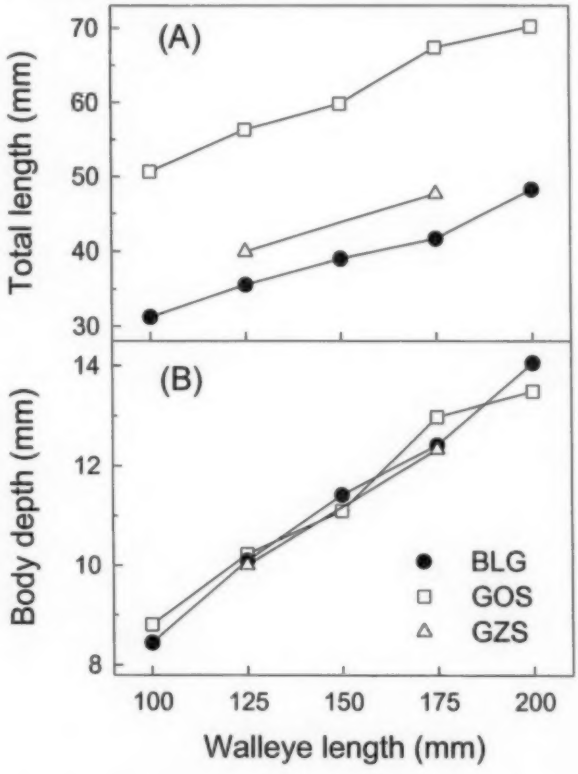

Fig. 2. Mean total length (A) and body depth (B) chosen by walleye size groups for bluegill (BLG), golden shiner (GOS), or gizzard shad (GZS) during size-selection experiments. For each prey and predator combination, at least 45 experiments were completed.

Results

Size selection

The size range of ingestible prey increased with walleye size (Fig. 1A). For each prey species, 100-mm walleye consumed prey over a 20-mm size range, whereas for 200-mm fish, this range was about 50 mm. For all prey species, selection peaked at intermediate prey sizes. The size range chosen also varied by prey species, being wider for golden shiner than for either gizzard shad or bluegill. Because of their fusiform shape, longer golden shiner (up to 55% of predator total length) than either gizzard shad (41%) or bluegill (38%) could be swallowed by all walleye sizes. Comparing selection based on body depth, walleye selected similar size frequencies regardless of prey species (Fig. 1B). We also compared mean total length

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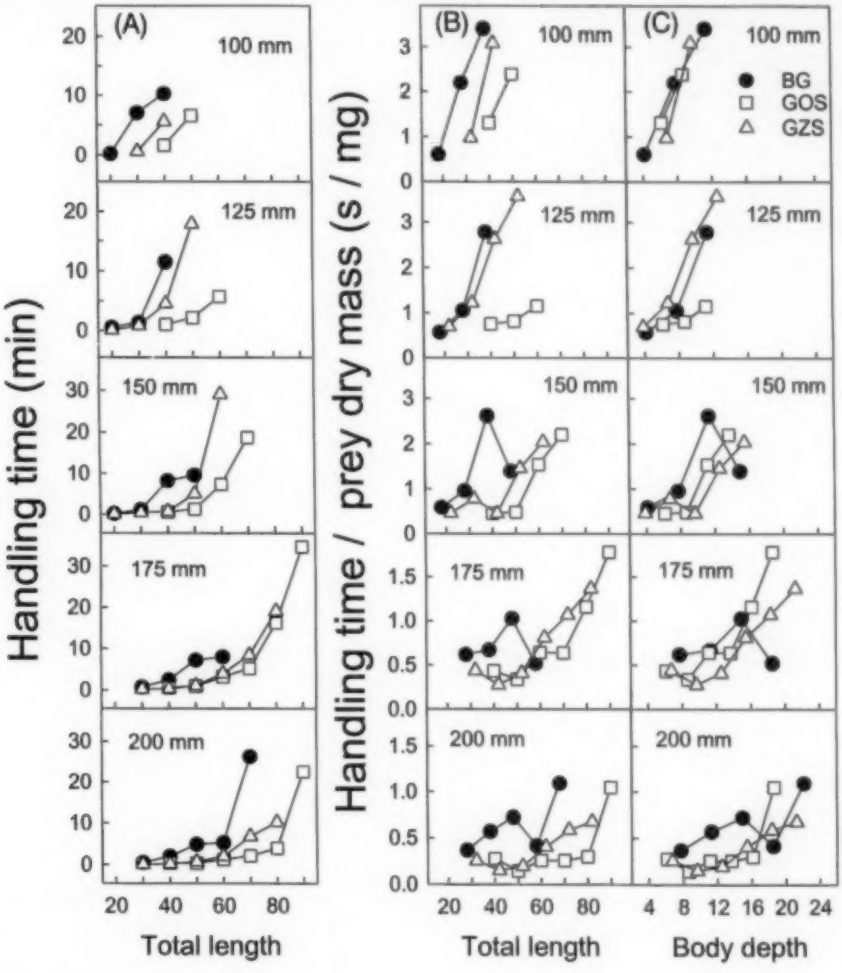
Fig. 3. Comparisons of mean handling time (A; min) and cost–benefit relationships (B and C; handling time/dry mass) for different sizes of bluegill (BLG), golden shiner (GOS), and gizzard shad (GZS) for each walleye size group. Prey sizes for cost–benefit relationships are presented for both total length (B) and body depth (C). At least 10 experiments were completed for each prey and predator combination.



and body depth of prey chosen by each walleye length class (Fig. 2). Although larger walleye could eat larger prey, sizes of prey chosen decreased relative to predator size. For example, 125-mm walleye consumed golden shiner averaging 44% of their body length, but for 200-mm walleye, this ratio was 35%. Walleye chose longer golden shiner than either gizzard shad or bluegill (Fig. 2A; ANCOVA, homogeneity of intercept, $P < 0.005$). However, body depths of prey chosen were similar for all three species (Fig. 2B; ANCOVA, homogeneity of intercept, $P > 0.29$).

Handling time increased with length at similar rates for all prey species (Fig. 3A; ANCOVA, log-transformed data, homogeneity of slope, $P > 0.17$). For similar-sized prey, handling times were highest for bluegill (ANCOVA, homogeneity of intercept, $P < 0.02$) but were similar between gizzard shad and golden shiner ($P = 0.11$). Handling times for bluegill were most variable and were influenced by prey capture location. For all sizes of walleye combined, bluegill captured in the tail region had higher handling times (mean 375 s) than similar-sized prey captured in the head (171 s) (paired t-test, $P = 0.02$). Smaller bluegill could be swallowed caudally, but larger bluegill (greater than 35% of walleye length) could only be consumed head first. As a result, larger bluegill often had similar or lower handling times than small bluegill ingested caudally. For 150–200 mm walleye, cost–benefit curves (handling time/prey dry mass) decreased for larger sizes of bluegill (50- and 60-mm size-classes), making it appear more profitable for walleye to capture and handle larger individuals (Fig. 3B). For gizzard shad and golden shiner, however, handling times were similar for equal-sized individuals regardless of capture location (paired t-test, $P > 0.08$). For all prey species, prey captured in the head region were larger than prey captured in the caudal region (ANOVA, $P < 0.01$).

We used handling time per prey dry mass ratios to determine optimal prey sizes for each walleye size group. Bluegill generated higher ratios, followed by gizzard shad and golden

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Fig. 4. Capture efficiency (captures per strike; %) as a function of prey length for all walleye size groups foraging on bluegill (BLG), golden shiner (GOS), or gizzard shad (GZS) during aquaria experiments.

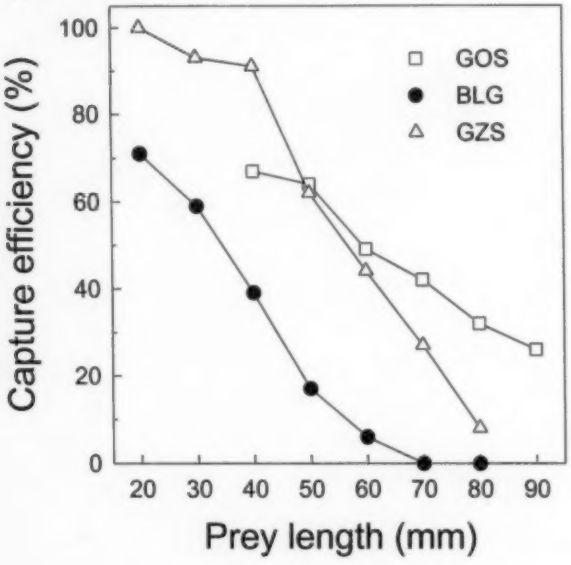
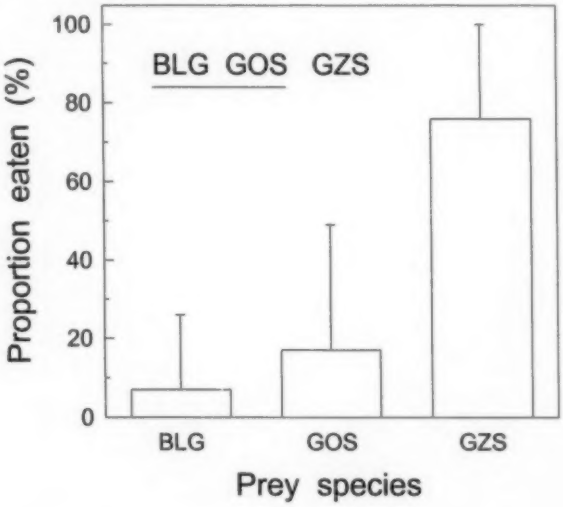


Fig. 5. Proportion eaten (%) of each species by walleye foraging on mixed species groups ($N = 5$ each) of bluegill (BLG), golden shiner (GOS), and gizzard shad (GZS) during pool experiments. Error bars are the upper 95% confidence limits. Underlined species are not significantly different from one another (ANOVA, df = 2, 12; Tukey's HSD, $P > 0.05$).



shiner (Fig. 3B). Optimal size for each species was determined as those ratios that did not differ significantly from the lowest value (ANOVA, LS means, $P < 0.05$). For all walleye size groups combined, optimal prey length ranges were smaller for bluegill (15–24% of predator total length (TL)) and gizzard shad (15–27%) than for golden shiner (26–39%). Based on prey body depth, however, optimal prey ranges were similar for all three species (4–8% of walleye TL; Fig. 3C). These optimal size ranges determined from cost–benefit curves were compared with sizes chosen by walleye.

Sizes most often selected were determined as those chosen in at least 20% of the trials for each walleye size-class. Walleye most often selected prey sizes toward the upper end of the optimal size range, and larger golden shiner (38–43%) were chosen compared with bluegill (20–32%) and gizzard shad (24–33%). Comparing body depth ratios, however, these ranges overlapped, with walleye most often choosing similar sizes of golden shiner (6–8% of walleye TL) as bluegill (5–9%) and gizzard shad (6–9%).

Capture efficiencies decreased with increasing prey size for all three species (Fig. 4). Compared as a function of the ratio of prey length to walleye length, walleye had higher capture efficiencies with gizzard shad as prey than with bluegill (ANCOVA, homogeneity of intercept, $P = 0.01$). Golden shiner were captured at rates similar to the other two species (ANCOVA, homogeneity of intercept, $P > 0.06$).

Species preference

During pool experiments, individual walleye captured from 1 to 5 prey, averaging 2.4 prey per experiment. Walleye captured more gizzard shad than either bluegill or golden shiner (Fig. 5; ANOVA, $F = 21.1$, df = 2, 12, $P < 0.001$; Tukey's HSD). Prey behavior differed among species prior to release of the predator; time spent in schools was highest for golden shiner (98%) and gizzard shad (96%), whereas bluegill rarely schooled (12%; ANOVA, $F = 171.9$, df = 2, 51, $P < 0.001$; Tukey's HSD). Golden shiner and gizzard shad often schooled together. In paired-choice experiments, walleye captured more gizzard shad than either bluegill or golden shiner (Fig. 6; chi-square, $P < 0.01$), whereas bluegill and golden shiner were captured in equal numbers (chi-square, $P > 0.50$).

Prey vulnerability

During behavioral observations, similar numbers of each prey species were captured per experiment (Table 1), but walleye experienced more difficulty capturing bluegill than either gizzard shad or golden shiner. Bluegill required twice as many follows and three times the number of pursuits as gizzard shad; golden shiner were intermediate but not different from the other two (Table 1). Walleye also made more strikes at bluegill compared with golden shiner and gizzard shad (Table 1). As a result, capture efficiencies were higher for gizzard shad (0.41) and golden shiner (0.32) than for bluegill (0.13) (ANOVA, $F = 11.85$, df = 2, 12, $P < 0.001$; Tukey's HSD).

Time spent foraging by walleye varied with prey species (Fig. 7). During 30-min experiments, total time spent in all behaviors leading to attack, capture, and ingestion was highest for bluegill (18.3 min), followed by golden shiner (12.6 min) and then gizzard shad (6.0 min; ANOVA, $F = 10.33$, df = 2, 12, $P = 0.02$; Tukey's HSD). Of this time, similar amounts were spent searching and observing for all three prey species (Fig. 7). However, the amount of time required for follow and pursuit, more energetically demanding behaviors, was higher for bluegill compared with gizzard shad, with golden shiner intermediate (Fig. 7).

Behavioral differences among prey species in response to

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Fig. 6. Proportion captured (%) for walleye offered all pairwise combinations of bluegill (BLG), golden shiner (GOS), and gizzard shad (GZS) in aquaria (72 L). *N*, number of experiments for each combination. Error bars are Clopper–Pearson 95% confidence limits. Values that are significantly different from one another are denoted by an asterisk (chi-square, $P < 0.05$).

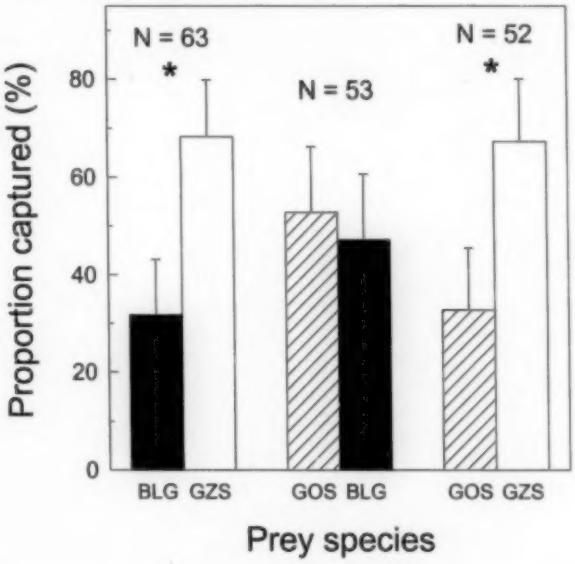

Table 1. Comparisons of prey and predator behaviors (mean ± 95% confidence interval) for walleye feeding on single species groups ($N = 15$) of gizzard shad, golden shiner, or bluegill during each 30-min experiment.

Behavior	Prey species: Gizzard shad	Bluegill	Golden shiner
Prey behavior			
Time schooled (%)	84±11.4*z*	73±7.7*x*	99±1.7*y*
Distance to walleye (cm)			
Closest schooled	75±9.2*ab*	877.3*bc*	98±8.5*c*
Closest dispersed	70±13.3*a*	70±6.9*a*	64±65.6*a*
Predator behavior			
No. captured	1.6±1.2*z*	1.3±0.5*z*	1.6±0.5*z*
No. of follows	5.2±2.0*z*	12.7±3.8*y*	8.1±2.5*yz*
No. of pursuits	5.5±1.9*z*	16.1±5.6*y*	8.4±2.4*yz*
No. of strikes	4.9±1.7*z*	14.6±5.1*y*	7.0±2.0*z*
Distance prey followed by predators			
Distance to prey (cm)	36±7.9*y*	56±10.4*z*	61±11.8*z*
Mean number of prey attacked			
Schooled	2.3±0.5*a*	5.0±5.1*b*	6.6±1.5*bc*
Dispersed	3.7±2.6*ab*	10.1±9.2*c*	1.8±1.5*a*
Capture efficiency (%)			
Schooled	26±0.2*ab*	13±0.1*a*	20±0.3*ab*
Dispersed	33±0*b*	13±0.1*a*	70±0.3*c*

Note: Row values (*x*, *y*, and *z*; ANOVA, Tukey's HSD) and row and column values (*a*, *b*, and *c*; two-way ANOVA, LS means) with the same letter are not significantly different ($P > 0.05$).

the presence of a predator affected their differential vulnerability (Table 1). All three prey species schooled the majority of time (Table 1), but golden shiner schooled almost exclusively, while bluegill schools often broke up and re-formed. Golden shiner schools remained farther from the predator than gizzard shad, with bluegill intermediate, which may explain longer following distances for golden shiner and bluegill compared with gizzard shad (Table 1). Although walleye attacked more schooled than dispersed golden shiner (ANOVA, $P < 0.001$), capture efficiencies were lower for schooled prey ($P < 0.01$), suggesting this was an effective antipredatory tactic for this species. Schooling may not be as critical for bluegill; although schools maintained greater distances from the predator (ANOVA, $P < 0.002$), and dispersed individuals were attacked more often ($P < 0.05$), walleye capture efficiencies were similar for dispersed and schooled bluegill ($P = 0.93$). Walleye were also equally successful capturing either schooled or dispersed gizzard shad (ANOVA, $P = 0.40$); both maintained similar distances from the predator ($P = 0.62$), and were attacked a similar number of times ($P = 0.23$).

Morphological differences among prey species, such as body depth and dorsal spines, did not influence predator capture location (Table 2). For optimal-sized prey of all three species, walleye most often captured prey in the caudal region rather than the head or midbody (chi-square, $P < 0.001$). After capture, bluegill were more difficult to ingest. Handling times for the first prey captured were highest for bluegill but similar between golden shiner and gizzard shad (Fig. 7).

Discussion

Prey morphology influenced size selection by walleye. Prey body depth is greatest for bluegill, followed by gizzard shad and golden shiner. As a result, walleye chose larger sizes of the fusiform golden shiner compared with deep-bodied bluegill and gizzard shad. However, body depths of ingested prey were similar for all prey species. Similar to other piscivores, walleye are gape limited and swallow prey laterally. Thus, body depth is a greater constraint than length for swallowing prey. Other studies have argued both size and species preference of predators can be better predicted by indices relating either prey body depth to predator mouth size (Gillen et al. 1981; Hambright 1991) or prey diameter (including spines) in proportion to mouth diameter (Reist 1980; Reimchen 1991). For juvenile walleye, body depth proved to be a better overall predictor than length for selection across prey species with differing morphologies.

Size ranges of prey consumable by juvenile walleye varied by species. As body depth increased, ranges of vulnerable prey narrowed. Differences in vulnerable size ranges among prey species may have important implications for growth and survival. Deep-bodied prey species can grow to a size refuge from predation faster than fusiform prey (Hambright et al. 1991), leaving fewer individuals vulnerable to predation in a centrarchid-dominated lake compared with a cyprinid system. These differences may partially explain variation in growth observed across systems with diverse forage. However, prey density is also important, as growth has been positively correlated with densities of vulnerable young-of-year bluegill (Santucci and Wahl 1993).

Capture efficiencies were inversely related to prey size. Walleye had difficulty grasping and holding larger prey, resulting in more strikes for a capture. We also expected that

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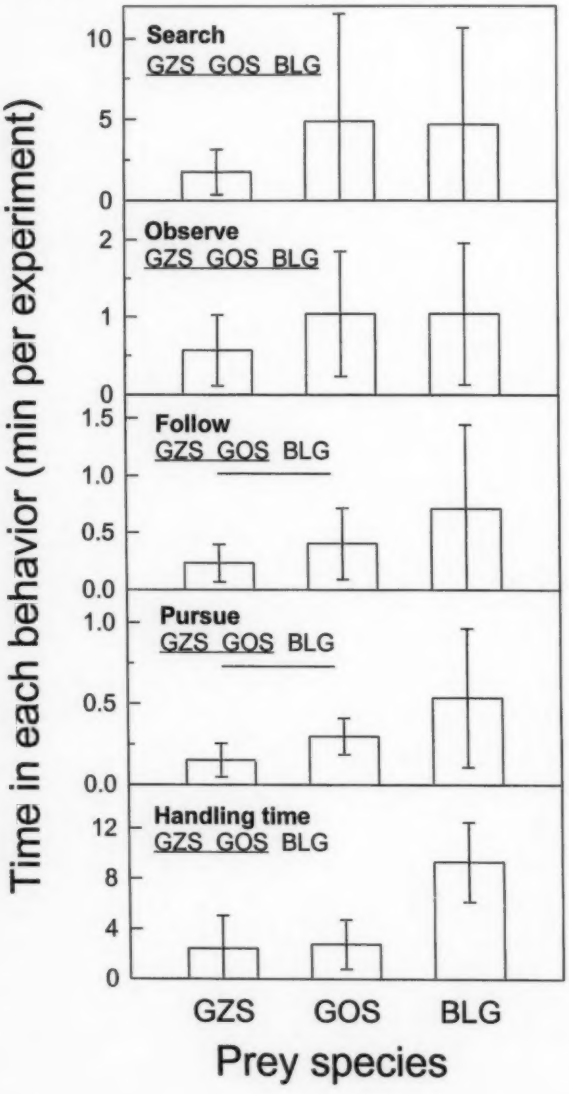
Fig. 7. Time spent in predatory behaviors by walleye foraging on bluegill (BLG), golden shiner (GOS), or gizzard shad (GZS) during 30-min tank experiments. Means were obtained from four to seven replicate experiments for five walleye. Error bars are 95% confidence intervals. Underlined species are not significantly different from one another (ANOVA, df = 2, 12; Tukey's HSD, $P > 0.05$).



capture efficiencies would be lower for deep-bodied prey (Webb 1986; Hambright 1991). Walleye had higher capture efficiencies for gizzard shad than for golden shiner, indicating other factors, such as prey behavior, are also important in influencing capture success. Other laboratory and field enclosure studies have also found deep-bodied prey with soft rays more vulnerable to predation than fusiform prey and have suggested that differences in capture rates are likely due to prey movement patterns and habitat use (Tonn et al. 1991; Greenberg et al. 1995).

Table 2. Capture location for walleye feeding on gizzard shad, golden shiner, or bluegill in tank experiments (750 L).

		Capture location (%)		
Prey species	*N*	Head	Midbody	Caudal
Gizzard shad	39	20z	21z	59y
Golden shiner	39	7z	13z	80y
Bluegill	32	19z	22z	59y

Note: Values with the same letter within a row, column, or both are not significantly different (chi-square, partitioned degrees of freedom, $P > 0.05$). Total number of captures (*N*) is given for each prey species.

Unlike other piscivores, which ingest prey head first (Juanes and Conover 1994), walleye often capture and swallow prey caudally (Wahl et al. 1995). Caudal captures are typical of predators that pursue rather than stalk prey (Popova 1978). However, large prey that were difficult to handle were captured in the head region, similar to other predators (Reimchen 1991). Handling times also differed with prey species. Similar to other piscivores, handling times were highest and most variable for bluegill (Gillen et al. 1981; Hoyle and Keast 1987; Wahl and Stein 1988), and were influenced by capture location. When captured caudally, bluegill extend their dorsal and anal spines as a defensive tactic, which either increased handling time or prevented ingestion, depending on prey size. Walleye were most efficient capturing and swallowing bluegill head first. In contrast, soft-rayed prey were easier to swallow, and neither capture location nor body depth influenced handling time. Gizzard shad, although deep-bodied, lack the spines and rigid body structure of bluegill. Walleye could slightly compress gizzard shad to facilitate swallowing, and handling times were similar to those for golden shiner. In work with other prey species, juvenile walleye also selected cyprinids over spiny-rayed yellow perch (*Perca flavescens*) (Lyons 1987). However, yellow perch were eaten more often than darters. High consumption of yellow perch may result from their having shorter spines than bluegill and a more fusiform body (Savitz and Janssen 1982).

Handling times increased at a greater rate and optimal size ranges were narrower for deep-bodied prey than for fusiform ones. However, prey sizes chosen by walleye were not fully explained by an optimal foraging model. Walleye chose prey sizes that were toward the upper end or larger than those predicted to be optimal. These results conflict with other studies, which have generally found prey consumed by several piscivores to be smaller than optimal (Juanes 1994; Paszkowski and Tonn 1994; Greenberg et al. 1995). Discrepancies between optimal size ranges generated in the laboratory and sizes observed in the field are likely related to prey availability. Our results may have been affected by the use of small aquaria, where differential effects of prey size could be minimized by limited opportunity to escape. Alternatively, optimal foraging models have been tested mainly for planktivores in which prey handling times are the primary constraint on prey capture. Piscivores generally capture large prey infrequently (Breck 1993) and may choose prey based on size rather than handling time. For piscivores, prey encounter rates may be more important

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than handling times, and different decision rules may be necessary to evaluate foraging decisions.

Walleye prey preferences were related to the amount of time and energy spent on capture. In pool and aquaria experiments, walleye chose gizzard shad most often, whereas bluegill were least preferred. Comparative laboratory studies with esocids have also found gizzard shad to be preferred prey over bluegill, with cyprinids intermediate (Mauck and Coble 1971; Wahl and Stein 1988). These results also correspond to preferences inferred from stomach contents for walleye in field studies (Knight et al. 1984; Johnson et al. 1988). Direct relationships probably exist between our laboratory prey preferences and growth and survival in the field (Wahl and Stein 1988; Santucci and Wahl 1993).

We found walleye preference of golden shiner intermediate to gizzard shad and bluegill. Golden shiner co-exist in lakes with large predators by outgrowing vulnerability to predation (Rahel 1984); however, we also observed antipredatory behaviors that could increase survival for vulnerable size-classes. Fathead minnow are often used as a model cyprinid in predator–prey studies, despite rarely co-occurring with large predators in lakes (Tonn and Magnuson 1982; Robinson 1989; Savino and Stein 1989) and high vulnerability to predation in laboratory studies (Moody et al. 1983; Wahl and Stein 1988). Golden shiner may be a more useful model for cyprinids in behavior studies as this species is better adapted to predation pressure.

Prey exhibited different antipredatory behaviors that affected preference by walleye. Foraging was more costly for walleye when bluegill were prey, requiring more follows, pursuits, and strikes per capture than for other prey. Bluegill engaged in flexible antipredatory behaviors similar to those described in the presence of other piscivores. Often bluegill would appear physically larger by orienting perpendicular to the predator and extending dorsal and anal spines, and evade attacks through back-peddling and dart and freeze escape (Savino and Stein 1982; Howick and O'Brien 1983; Moody et al. 1983; Wahl and Stein 1988). Using these behaviors, bluegill could avoid capture whether they were dispersed or schooled. Other prey mainly used schooling as a defense. Schooled prey remained vigilant of walleye and often moved away from the predator to reduce the probability of attack. Among prey species, schools differed in distances they maintained from the predator. Similar to esocids (Wahl and Stein 1988), walleye could get closer to gizzard shad than to other prey. Predator awareness influences the speed at which prey initiate escape when attacked, and prey with slower escape responses are more likely to be captured (Webb 1986). Proximity to the predator, combined with a slower response when attacked, may have increased vulnerability of gizzard shad to predation.

We examined several important components of prey size and species selection by juvenile walleye. Prediction of optimal size in piscivores will require incorporation of components of foraging other than handling time. Unlike previous conclusions for other piscivores (Juanes 1994), our results suggest active prey selection by walleye. Similar to other piscivores, walleye prey species preferences can be explained based on time and energy spent foraging. Across piscivores with differing predatory strategies, gizzard shad are preferred over cyprinids and bluegill (Wahl and Stein 1988; this study). Behavior and morphology, unique to each prey species, influences vulnerability to predation.

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Channel morphology and lateral stability: effects on distribution of spawning and rearing habitat for Atlantic salmon in a wandering cobble-bed river

Brigid A. Payne and Michel F. Lapointe

Abstract: Pronounced downstream variations in channel morphology in the wandering, gravel–cobble Nouvelle River, Quebec, provided an opportunity to assess certain effects of channel planform and stability on rearing and spawning habitat for Atlantic salmon (*Salmo salar*). At summer low flows, weighted useable area per unit channel length differed significantly among five reaches of contrasting morphology with identical discharge regimes. A braid-like reach, dominated by a wide, dissected midstream bar, offered three to five times more potential habitat for juveniles (21 m usable width for fry, 12 m for parr) than two sharply curved reaches with minor backchannels (4 m for both fry and parr) and two to three times more potential habitat than two moderately curved reaches. Fining of potential spawning riffles downstream from eroding cutbanks was not detected, in five unstable river reaches even where erosion rates reached 10 m/year, and the percentage sand content of eroding banks was five times that of instream gravels. This finding challenges the assumption that large local inputs of sand necessarily cause fining of instream spawning gravels, and suggests that hydraulic forces in a moderately powerful river can be quite efficient in preventing the buildup of excess fines in midchannel riffle habitat.

Résumé : Des variations prononcées en direction de l'aval dans la morphologie du chenal de la rivière Nouvelle au Québec, une rivière à lit de graviers et cailloux, nous a permis d'évaluer certains effets de la forme en plan et de la stabilité du chenal sur l'habitat d'alevinage et de fraye du saumon atlantique (*Salmo salar*). Aux débits d'étiage d'été, la superficie utilisable pondérée par unité de longueur de chenal était significativement différente entre cinq tronçons à morphologie contrastante mais régime hydrologique semblable. Un tronçon «réticulé», dominé par un large banc disséqué en plein milieu du chenal, offrait de trois à cinq fois plus d'habitat potentiel pour les juvéniles (largeur utilisable de 21 m pour les alevins et de 12 m pour les tacons) que deux autres tronçons présentant des courbes prononcées et des petits chenaux secondaires (4 m pour les alevins et les tacons) et de deux à trois fois plus d'habitat potentiel que deux tronçons modérément recourbés. Nous n'avons pas décelé d'affinement des graviers sur les bancs de fraye dans cinq tronçons instables, en aval des berges en cours d'érosion, même là où les taux d'érosion atteignaient 10 m/an et la teneur en sable des berges en cours d'érosion était de cinq fois supérieure à celle des graviers du lit. Ces résultats infirment l'hypothèse selon laquelle d'importants apports locaux de sable entraînent obligatoirement une détérioration du substrat de fraye. Ils suggèrent que les forces hydrauliques d'un cours d'eau de puissance moyenne peuvent empêcher de façon efficace l'accumulation de particules fines dans le substrat des bancs de fraye en mi-chenal.
[Traduit par la Rédaction]

Introduction

A balanced distribution of both spawning and rearing habitat is needed to ensure maximum salmon production from a reach of river. Both these habitats are strongly controlled by the geomorphic processes that determine local channel morphology, hydraulics, and substrate character, as well as by other biotic and abiotic variables. In this paper, we investigate a number of these geomorphology habitat interactions.

Like many riverine organisms, juvenile Atlantic salmon (*Salmo salar*) require a certain range of depths and current velocities for optimal growth and survival. Hogan and Church (1989) termed this range the "hydraulic habitat" of a species. Certain environmental variables, such as discharge, valley gradient, and sediment supply, control mean channel morphology and hydraulics and, therefore, influence the availability of hydraulic habitat in rivers (Binns and Eiserman 1979; Platts 1979; Lanka et al. 1987). Factors such as local valley constrictions, local changes in channel slope and bank erodibility, and point sources of sediment introduce local variability to the channel planform and, therefore, to the hydraulic habitat available at the reach scale. The link between planform, hydraulics, and fish habitat is widely recognized (Platts 1979; Bisson et al. 1982; Beschta and Platts 1986; Frissell et al. 1986; Heede and Rinne 1990; Rabeni and Jacobson 1993), but few systematic, quantitative evaluations of the effect of river planform on hydraulic habitat availability have been reported.

The quality and stability of spawning substrate is a second key concern for managers wishing to preserve salmonid habitat (Adams and Beschta 1980; Beschta 1987; Lisle 1989).

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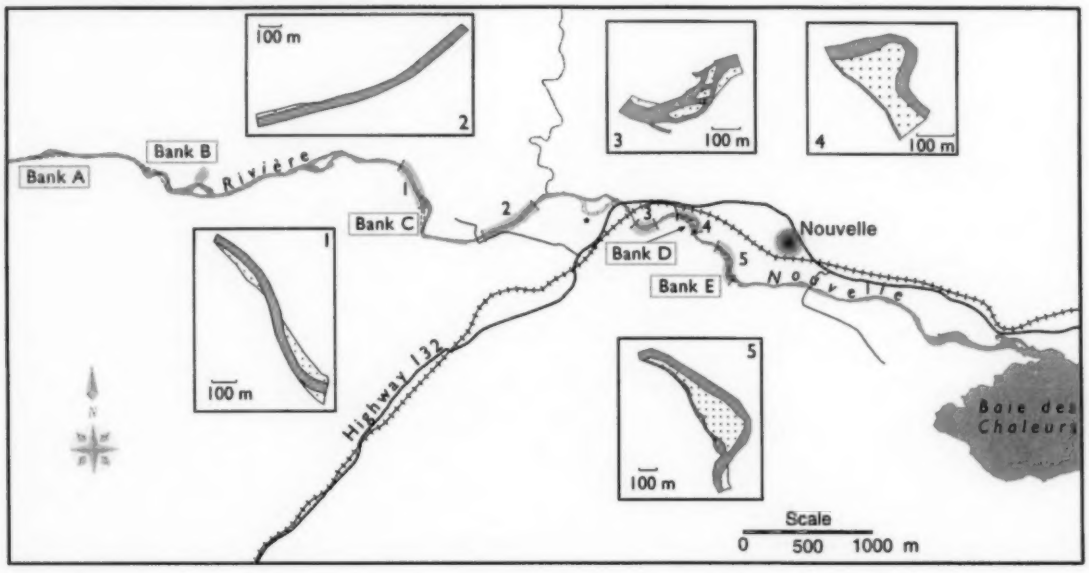
Fig. 1. The Nouvelle River in the Baie des Chaleurs region of Eastern Quebec, Canada. Insets show plan morphology of study reaches 1–5. Dotted channel sections denote 1981 locations of eroding bank sections A–E. The asterisk near Highway 132 refers to a recent channel avulsion discussed in the text (*river path pre-1981).



Excess fine sediments in spawning gravels can lower salmonid survival by inhibiting the supply of oxygen to, and the removal of metabolites from, salmon embryos developing in the interstices of the gravel bed (McNeil and Ahnell 1964). Emergence of salmon fry from the gravel bed may also be mechanically blocked if the gravel substrate is not sufficiently coarse (Phillips et al. 1975; Hausle and Coble 1976). Excess fines in a channel may arise from a variety of sources. Road building and forest clearing produce fines, which can accumulate in the bed substrate and reduce spawning success for salmonids (Platts and Megahan 1975; Phillips et al. 1975; Everest et al. 1985; Scrivener and Brownlee 1989). As well, prolonged bank erosion sometimes occurs following channel rectification (artificial straightening of a channel) as the channel reverts to an equilibrium level of curvature (Brookes 1985). Such erosion can increase downstream deposition of fine sediments to the detriment of fish habitat (Little 1973). High rates of bank erosion are generally a serious concern for local fisheries managers.

In this paper, we investigate certain effects of reach-scale variability in channel stability and morphology on spawning and rearing habitat for salmonids along a moderately high-energy, gravel–cobble river. Two major questions are addressed. First, at a given flow level, how does juvenile salmon habitat availability (measured according to published criteria) vary in a river that has strong between-reach contrasts in channel pattern? Second, do rapidly eroding, sand-rich cutbanks cause a noticeable increase in the fine content of downstream spawning gravels?

Study site

The Nouvelle River is one of several gravel–cobble rivers flowing through the Southern Gaspé Peninsula of eastern Quebec (Fig. 1). Resource managers are currently stocking the river with Atlantic salmon (*Salmo salar*), and this, in part, motivated our assessment of salmon habitat in the lower, unstable reaches of the river. Unlike in neighbouring rivers, there is no evidence of an historic salmon population in the Nouvelle River, although there is a resident population of sea-run brook trout (*Salvelinus fontinalis*). Since Atlantic salmon is currently absent from this system, the study focuses on the relations between channel form and the availability of physical habitat, as defined through published habitat preference criteria for Atlantic salmon.

The Nouvelle River arises at an elevation of 600 m in the Chic-Choc Mountains and drains an area of 1200 km^2 into the Baie des Chaleurs. Stream flow is unregulated and subject to large seasonal fluctuations. The mean annual discharge in the lower 18 km where we collected data is 26 m^3/s, and channel gradients range from 0.0015 to 0.0032 m/m. Peak flows occur during the spring freshet and the peak daily flow with 2-year recurrence is 260 m^3/s. A typical summer low flow of 24 h duration and 2-year recurrence is 6 m^3/s. The unit stream power (ω) measures the rate at which water flowing downstream expends energy on a unit area of bed during channel-forming flows and is a function of the density of water (ρ), the acceleration due to gravity (g), bankfull discharge (Q), channel gradient (S), and the channel width (W):

$$\omega = \rho g Q S / W$$

In the lower, study reaches of the Nouvelle River, the hydraulic power of bankfull flows varies from 70 to 100 W/m^2, which is exerted on a cobble substrate with a median particle diameter of 4–5 cm. Ferguson (1987) classified rivers with unit stream

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Table 1. Planform characteristics of study reaches in the Nouvelle River.

Reach	SR_{max} (m/year)	W_b (m)	W/D_{Q1}	W/D_{Q2}	r_c/W_b	Channel pattern description
1	4	44	56	65	9.3	Single channel, moderate curvature, moderate shifting
2	0	43	72	91	34.9	Single channel, low curvature, laterally stable
3	9	103	130	108	3.5	Wide bend, dominant dissected mid-stream bar, rapid shifting
4	10	72 (21)	41[a]		1.2b	Twin channel, extreme curvature, rapid shifting
5	5	61 (20)	50	35	2.9[b]	Twin channel, strong curvature, moderate shifting
Mean	5.6	65	77[c]	75[c]	10.4	

Note: SR_{max} is the shifting rate at the point of maximum bank attack in each reach. W_b is the combined bankfull width of all major and minor channels in a reach; values in parentheses are backchannel widths at bankfull. W/D_{Q1} and W/D_{Q2} are the ratios of average, total wetted width to average flow depth at $Q1$ and $Q2$ discharges (see text). Curvature is measured by r_c/W_b, where r_c is the centre-line radius of curvature.

[a] Reach 4 was surveyed at an intermediate discharge of 18 m^3/s.

[b] Main channel only.

[c] Mean excludes reach 4.

powers in this range as moderately high energy and likely to display well-developed sinuosity and active lateral shifting.

More precisely, the lower Nouvelle River displays "wandering" characteristics, i.e., its channel pattern is transitional between meandering and braiding (Church 1983). In such rivers, variations in sediment supply and bank erodibility cause strong inter-reach contrasts in morphology and stability; straighter, more stable reaches alternate with reaches showing varying degrees of bar development, curvature, and channel splitting. In the lower Nouvelle River, bank erosion rates range from near zero in stable reaches to 5–10 m/year in zones of active lateral shifting. These latter, unstable zones are in some measure due to the progressive reestablishment of an equilibrium level of curvature following channel rectification projects in the 1960s (Gidas 1976; Brookes 1985). Also, aerial photographs indicate that, at some point in the 1980s, the main channel of the river switched courses, cutting through approximately 500 m of cleared floodplain (Fig. 1). The large volumes of sediment released downstream by this channel avulsion are a further cause of channel instability in some reaches of the Nouvelle River.

The lower Nouvelle River is bordered by floodplain and low terraces, which are covered by a mixture of riparian forest and cleared pasture. The top stratum of cutbanks is generally 0.2–1.5 m thick and composed mainly of sand. Underlying this layer there is a stratum, on average 1 m thick, of mixed sand and gravel, followed by a coarse gravel and cobble stratum extending down to the elevation of the thalweg (the deepest point in a channel cross section). While the average sand content in the cobble substrate of the low-flow channel is under 10%, average sand content in active cutbanks is substantially greater, ranging from 30 to 60%. Rapidly retreating bank sections therefore release substantial volumes of relatively fine material to the channel.

Methods

Hydraulic habitat for juveniles

From aerial photographs and field visits, we selected five river reaches with strongly contrasting morphologies (Table 1; insets of Fig. 1) among which to compare hydraulic habitat availability for juveniles. One minor tributary, the ruisseau Mann, enters amid the study area increasing summer flows by less than 10%, a negligible contribution with regard to our hydraulic comparisons among reaches. Thus, to a very good approximation, all study reaches are formed by the same flow regime and inter-reach habitat differences purely reflect the contrasts in local stream morphology.

In each reach, measurements of depth and mean water-column velocity were taken along transects placed one to two channel widths apart. Readings were spaced so that each point represented the depth and velocity conditions of a hydraulically uniform area of river, here termed a habitat cell. Consequently, the density of readings was greatest in hydraulically complex areas. Velocity was measured at a distance from the surface equal to 60% of the total depth with a Price meter fixed to a top-setting wading rod. The size composition of gravels on the bed surface was estimated visually for each habitat cell because, in addition to depth and velocity, the size of particles on the bed surface is a determinant of habitat usability (Gibson et al. 1990; Gibson 1993). In 10 calibration tests, visual estimates of the median particle size at the bed surface (D_{50}) differed by ±1 cm from Wolman sample estimates in which the diameters of 100 particles were measured (Wolman 1954); visual estimates of the diameter below which 90% of particles fall (D_{90}) were within 2 cm of Wolman sample estimates.

Natural fluctuations in discharge can confound inter-reach comparisons of hydraulic habitat availability. Fortunately, in the summer of 1994, climatic conditions produced two extended periods of relatively uniform flow in the Nouvelle River, so that the study reaches could be surveyed under similar discharges. Between July 19 and August 3, depth and velocity surveys were conducted in reaches 1, 2, 3, and 5 at an average discharge of 24 m^3/s (henceforth called $Q1$). $Q1$ corresponds to average June flows in this river. The same four reaches were surveyed again between August 18 and 31 at an average discharge of 8 m^3/s ($Q2$). This latter discharge approaches the mean summer low flow with a 2 year recurrence of 6 m^3/s. In reach 4, time constraints allowed only one survey on July 28 and 29 at a midsummer discharge of 18 m^3/s.

We compared hydraulic habitat availability among reaches and between discharges ($Q1$and $Q2$) in two ways. First, the hydraulic characteristics of each study reach were examined qualitatively using bivariate distributions of depth and velocity conditions (by area of bed) from hydraulic survey data. Contours corresponding to the depth and velocity ranges of habitats commonly chosen by juvenile Atlantic salmon (Morantz et al. 1987) were superimposed on distributions to illustrate the potential hydraulic suitability of each reach. Second, weighted useable areas (WUA) were calculated according to the method of Bovee (1982). Under this technique, each habitat cell was multiplied by a "probability-of-use" (POU) factor, then weighted areas were summed to produce a reach WUA estimate. POU was calculated as the product of suitability indices for depth and velocity alone, as substrate was suitable throughout the study reaches. Since our study goal was to measure hydraulic suitability only, no attempt was made to quantify other aspects of habitat, such as cover or food supply. Ideally, suitability indices should be drawn from observations of

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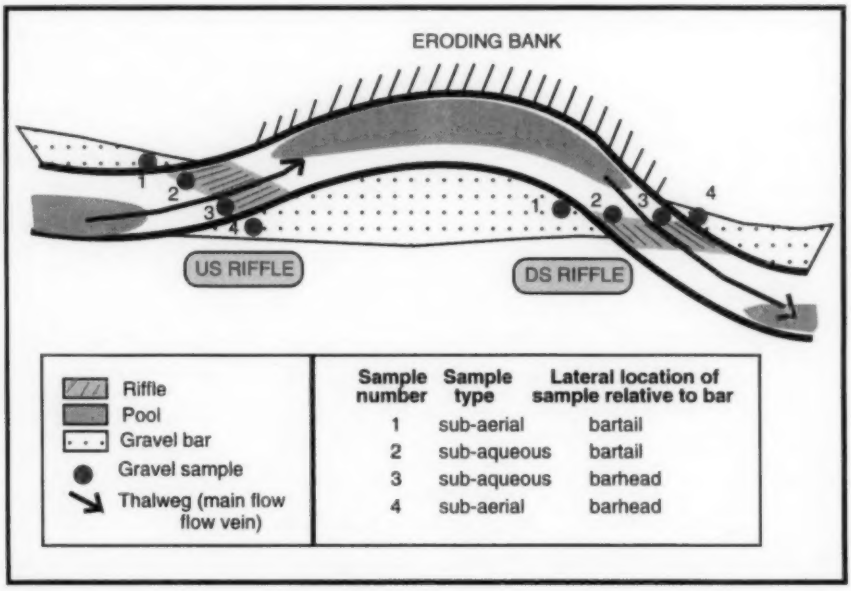
Fig. 2. Schematic diagram of cutbanks, riffles, and generic substrate sampling locations 1–4. Differences in sand content between upstream (US) and downstream (DS) samples from the same generic location were used to test the effect of eroding banks on riffle substrate composition.



Sample number	Sample type	Lateral location of sample relative to bar
1	sub-aerial	bartail
2	sub-aqueous	bartail
3	sub-aqueous	barhead
4	sub-aerial	barhead

habitat use by salmon in the study river itself. However, such observations were impossible in the Nouvelle River because of the absence of a resident salmon population. Instead, we used habitat preference curves from Morantz et al. (1987) that summarize summer habitat use by juvenile Atlantic salmon in six eastern Canadian streams (the largest of which is comparable in size to the Nouvelle River).

In quantitative, among-reach comparisons of habitat availability, WUA was normalized by reach length (AUW (average useable width) = WUA per metre channel length) and by overall area (PUA (percent useable area) = WUA divided by the total surface area of the wetted channel at a given flow).

Effects of bank erosion on spawning substrate

Sequential aerial photos from 1981 and 1992 were used to identify five bank sections in the lower 10 km of the river with high rates of lateral migration (Fig. 1). From these, we selected eight eroding cutbanks flanked upstream and downstream by potential spawning riffles. To compare fines content, substrate samples were extracted, upstream and downstream of the cutbank, along transects placed a short distance upstream of the riffle crest in the zone most commonly used for salmon spawning. The two consecutive eroding sections in each of bank sites B, D, and E (Fig. 1) were treated separately; the single riffle separating these cutbank sections served simultaneously as a downstream and upstream sample location to the respective cutbanks. To control for the typically strong pattern of lateral variability in substrate composition along channel cross sections (Lisle 1989), substrate from four generic locations were sampled along each transect (Fig. 2). Two samples, one sub-aerial and one sub-aqueous, were extracted from the "barhead" side of the channel, i.e., the side submitted to strong flow velocities during formative flows (sample positions 3 an 4, Fig. 2). Similarly, a sub-aerial and a sub-aqueous sample were taken from the "bartail" or low-velocity side of the channel (sample positions 1 and 2). Sub-aqueous samples were located to evenly flank the thalweg. In some locations, sampling of all four locations was impossible because of excessive flow velocity in the channel, or because only one side of the channel was flanked by an exposed lateral bar.

All substrate sampling was conducted at low flow between August 13 and September 1, 1994. Discharges ranged from 7 to 15 m^3/s, which is less than the mean discharge for October (23 m^3/s), the month during which Atlantic salmon spawning commonly occurs in Gaspé rivers. The sub-aerial samples (Fig. 2, locations 1 and 4) were extracted as close as possible to the late-summer water's edge. These sites would thus typically be submerged and potentially accessible to adult salmon during the spawning season. In total, 41 substrate samples were extracted in the vicinity of the eight cutbank sections.

Substrate extraction and analysis

Volumetric samples in sub-aerial sites were extracted with a shovel according to conventional bulk-sampling techniques (Church et al. 1987; Lisle and Madej 1992; Lisle 1995). Sample holes were 25–30 cm deep, similar to the published depth of salmon spawning redds (Crisp and Carling 1989; Gibson et al. 1990). The average mass of sub-aerial substrate extracted was 48 kg. In sub-aqueous sites (Fig. 2, locations 2 and 3), we prevented the escape of fines during bulk sampling by excavating samples with a shovel within the confines of a flow-isolation chamber, 60 cm in diameter and 75 cm in height, which was driven several inches into the gravel bed. Affixed to this cylindrical cell were a water-permeable panel facing upstream and a conical net facing downstream, both constructed of 100-μm Nytex mesh. Water currents passing through the front mesh panel carried any fines thrown into suspension during digging into a cup at the end of the downstream net. After the substrate was extracted, the cup contents were collected and added to the sample. The average mass of sub-aqueous samples was 38 kg. For both sub-aqueous and sub-aerial samples, fractions coarser than 32 mm were sorted in the field using templates; finer fractions were then sieved to 16 mm. The remaining material (<16 mm) was split according to the technique of Church et al. (1987), and a subsample was brought back to the laboratory for sieving at half-phi intervals.

Church et al. (1987) recommend that, for statistical accuracy,

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sample size should be set such that particles in the largest size-class make up less than 1% of the sample by mass. For statistical analyses of gravel composition, we removed all particles larger than 64 mm so that all but four samples met the 1% criterion for mass (at 64 mm, the minimum sample required is 35 kg).

Results

Hydraulic habitat for juveniles

In all study reaches, visual estimates of D_{50} fell between 4 and 5 cm, and estimates of D_{90} lay between 9 and 13 cm. These results indicate that bed surface particles in the Nouvelle River are within the broad range of gravel–cobble substrate sizes preferred by juvenile Atlantic salmon (Morantz et al. 1987; Gibson et al. 1990). Thus, for simplicity, we excluded substrate from subsequent comparisons of habitat among reaches.

It is well known that a periodic sequence of pools and riffles form in straight, alluvial channels with active substrate transport (Dietrich 1987; Ferguson 1993). As meanders appear in a straight channel, bars become more pronounced, the vertical amplitude of the pool–riffle sequence grows, and bank erosion increases (Dietrich 1987). In highly erodible channels exposed to strong hydraulic forces (high stream power, ω) and large sediment supply, a multiple-channel, braided pattern commonly emerges. In these channels, the classic, periodic arrangement of pools and riffles breaks down, and a more chaotic pattern of pools and shoals appear, corresponding to areas where flows converge and diverge, respectively (Ashmore and Parker 1983; Ferguson 1987). Our data show how these contrasts in channel pattern and bed forms influence the spatial distribution of depth and velocity conditions in the Nouvelle River (Fig. 3).

Reach 1 has a regular, moderately curved planform (r_c/W_b = 9.3; Table 1) with well-developed pools and riffles (inset of Fig. 1) and considerable outer bank erosion (lateral shifting rates reach 4 m/year; Table 1). The lobes in the upper left of both the $Q1$ and $Q2$ depth–velocity distributions (Figs. 3A and 3B) correspond to fast flow over shallow riffles; the lobes in the middle of the panel correspond to pools; and the portion of the distribution falling near the origin represent the shallow, low-velocity edge areas most prevalent at the inner sides of bends. The superimposed habitat preference contours for fry (Fig. 3; shown only on left column) and parr (shown only on right column) coincide mainly with these edge areas. In this moderately high-energy reach, velocities are excessive in most riffle areas (Fig. 3,) and pools are too deep to provide habitat in the preferred ranges defined by Morantz et al. (1987), even at $Q2$, the lower discharge.

Reach 2 is the straightest of all study reaches (r_c/W_b = 34.9, Table 1) and has a slightly coarser bed than other reaches. The vertical amplitude of its pool and riffle sequence is relatively small, and much of the reach displays the uniform depths and velocities (Figs. 3C and 3D) characterizing a "run" (i.e., the flat, transitional zone between a pool and a riffle). The effects of this morphology on habitat are twofold. First, while the width of reach 2 is similar to that of reach 1 (reach 2, W_b = 43 m; reach 1, W_b = 44; Table 1), the pools in the straighter reach 2 are shallower. Cross sections in reach 2 thus have a higher width/depth ratio (reach 2, W/D_{Q2} = 91; reach 1, W/D_{Q2} = 65; Table 1) and, therefore, wider shallow edges than reach 1. Compared with reach 1, there is a greater relative frequency (by area) of depths under 30 cm in reach 2 (Fig. 3), and more of the shallow habitats meeting the Morantz et al. (1987) depth–velocity criteria for fry. Second, the absence of well-developed bars, bends, and bedforms reduces flow resistance and allows midchannel velocities to exceed those seen in reach 1; in areas of reach 2 deeper than 50 cm, velocities at $Q1$ generally exceed 0.75 m/s, at $Q2$, 0.60 m/s (Fig. 3). According to the curves of Morantz et al. (1987), parr habitat lies largely at depths greater than 50 cm and is, therefore, limited by excessive velocity in reach 2.

Reach 3 is composed mainly of a wide, fast-flowing run on the upstream side of a massive, dissected, midstream bar. One deep, narrow pool lies against the right channel bank downstream of the riffle face (Fig. 1). The overall shallow depths and complex pattern of gravel deposition in this reach lead to an irregular depth–velocity distribution with few areas deeper than 100 cm (Figs. 3E and 3F). This particular reach morphology has two major effects on habitat. First, shallow depths increase potential habitat for juveniles, because preferred habitat as defined by Morantz et al. (1987) is found in the low range of depths available in the Nouvelle River during the summer. Second, the massive gravel accumulation in reach 3 greatly widens the channel (W_B = 103; Table 1) and increases the total surface area available to juvenile fish. The overall shape of the depth–velocity distribution of reach 3 undergoes a greater change between $Q1$ and $Q2$ than the distributions of reaches 1 and 2. The weirlike hydraulic control exerted by the crest of the large, midstream bar in reach 3 is responsible for this stage effect. At $Q2$ (Fig. 3F), except for a few, fast-flowing spillways down the face of the riffle (appearing as lobes in the upper left of the plot) and an almost stagnant pool along the right riffle margin (the lobe in the lower right), the reach is predominantly slow flowing and shallow. This weir effect further enhances the hydraulic suitability of reach 3 for fry by ensuring slow flows throughout most of the reach.

In reach 5, a shallow, ephemeral back-channel produces limited, hydraulically suitable habitat at $Q1$. This habitat appears as a small lobe near the origin of this reach's depth–velocity distribution (Fig. 3G). By $Q2$, the back-channel dries up, and the corresponding lobe disappears (Fig. 3H). The highly curved main channel of reach 5 is narrow (Table 1). Pools and riffles are not as well defined in reach 5 as in reaches 1 and 2, and the proportion of the channel area deeper than 75 cm is greater in reach 5 than in reaches 1, 2, or 3. The resulting uniformly deep, narrow channel cross section of reach 5 provides little habitat within the preferred depths and velocities (Fig. 3).

Reach 4, surveyed at a flow between $Q1$ and $Q2$, falls into the same general planform category as reach 5. Both reaches have highly curved, narrow main channels and a minor back channel, which dries up in midsummer (Fig. 1). The depth–velocity distributions of reach 4 (Fig. 3I) and reach 5 (Figs. 3G and 3H) reflect this similarity. Both reaches have a relative scarcity of depths below 50 cm (excluding the back-channel in reach 5), an abundance of depths over 75 cm, and a lack of distinct lobes representing pools and riffles.

In our quantitative comparisons of WUA, we found notable differences among reaches (Table 2). Because all reaches are exposed to approximately the same flood regime and daily flows, these differences reflect the importance of reach-scale geomorphological processes on hydraulic habitat. Habitat differences were greatest at $Q2$, reflecting the tendency for

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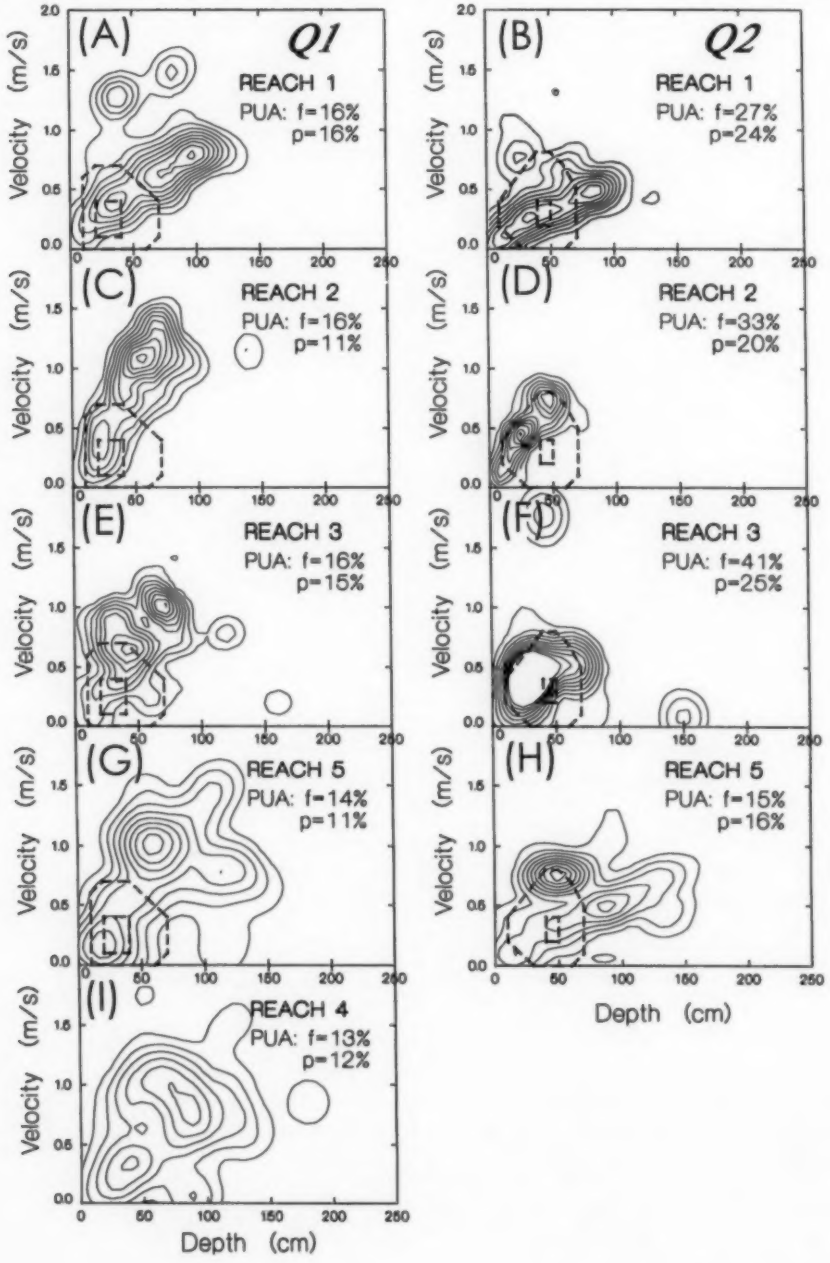
Fig. 3. Joint probability distributions of flow depths and velocities in each study reach, generated using a Kernel smoothing function (Silverman 1986). Left panels are for $Q1$ (24 m^3/s) conditions; right panels are for $Q2$ (8 m^3/s) conditions. Reach 4 was sampled at an intermediate discharge of 18 m^3/s. Solid contours depict the relative abundance of bed area in each depth–velocity class at intervals of 2.5% of the total reach wetted area. Peaks in the distribution represent the most widespread combination of depth and velocity in each reach. Broken contours correspond to probability of use (POU) values of 0.8 (inner line) and 0.2 (outer line) for fry (shown only on left panels) and parr (shown only on right panels) (Morantz et al. 1987). In each plot the percent weighted useable area (PUA) is given for both fry (f) and parr (p).



hydraulic variations induced by river form to increase with falling stage (Dietrich 1987). For instance, at $Q2$, useable width for fry (AUW_{fry}) in reach 3 was 21 m (Table 2), twice the average for all reaches and five times that found in reach 5 where AUW_{fry} equaled only 4 m (Table 2). In reaches 1 and 2, shallow habitats at the inner side of bends adjacent to bars offset unsuitable deep, fast, midstream areas to produce intermediate AUW values (Table 2).

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Effects of bank erosion on spawning substrate

The eight active cutbank sections selected to study the effect of fine sediment inputs on spawning gravels (Fig. 1) had an estimated 30–60% sand content by weight and eroded laterally between 3.3 and 10.5 m/yr (Table 3). One 260-m cutbank section (D.2) injected an estimated 1310 t/year of sand to the channel.

Sub-aerial samples from bar locations were generally finer than sub-aqueous samples taken from the wetted channel (Fig. 4; also, mean visual D_{50} estimate for sub-aerial samples is 16.1 mm, for sub-aqueous samples, 21.1 mm). This pattern of fining with increased elevation above the thalweg is common in gravel-bed channels (Lewin 1978). Among sub-aerial samples (Fig. 2), those extracted from the bartail side (where flood velocities are lower) had a higher fines content than those extracted from the barhead (higher flood velocities) side of the channel (Fig. 4). Again, this pattern of lateral fining is commonly observed in gravel-bed rivers (Lisle 1989). The systematic effects of sample location on gravel composition in riffle transects dictated how we tested for fines enrichment downstream from eroding cutbanks. Samples from the same generic sample location (Fig. 2) taken upstream and downstream of each eroding bank were matched, and pairwise comparisons of the fines content of these upstream–downstream pairs were conducted (Table 3). Only 5 of 15 sample pairs showed an actual downstream increase in their <2 mm fraction (Table 3). Least-squares regression of the change in fines content in upstream–downstream riffle pairs against bank sand input appears to suggest an overall enrichment effect for coarse sands (2-mm fraction) and fine gravel (4-mm fraction; Fig. 5); however, the positive trends are due mainly to one or two isolated points and are not significant.

Pairwise *t*-tests on the same upstream–downstream sample pairs used in Table 3 failed to show that upstream gravels differed from downstream gravels in fine content (Table 4, trial A, $P > 0.49$ for all fines fractions). A pooled subset of sub-aqueous samples (Fig. 2, locations 2 and 3 combined) also revealed no significant change in sand content downstream from highly erosive banks (Table 4, trial B).

Overall, only 4 of the 41 gravel samples collected from riffle gravels had sand compositions outside of optimal ranges for spawning success (i.e., survival to emergence) according to either the Fredle index (<6.5) of Lotspeich and Everest (1981) or the sand index (>1.5) of Peterson and Metcalfe (1981). According to these criteria, all of the sub-aqueous substrate samples (locations 2 and 3) were highly suitable for spawning, even those riffle samples taken downstream from the banks undergoing the greatest rates of erosion. Mean sand content for the submerged riffle samples was only 9%, whereas Peterson and Metcalfe (1981) observed that the emergence of Atlantic salmon fry from gravel substrates fell significantly only when substrate sand content exceeded 20%.

Discussion

The importance of planform to hydraulic habitat for fish has been recognized by several authors. Mosley (1982), in his study of the braided Ohau river in New Zealand, showed that, in the multiple channels of variable width and depth that characterize braided rivers, suitable hydraulic conditions for juvenile fish are maintained over a wider range of discharges than in a single-thread channel. Beschta and Platts (1986) discuss the difference in hydraulic habitat for fish between straight and meandering reaches. Restoration projects often call for the introduction of meanders into previously straight rivers to foster pool–riffle development, to increase hydraulic diversity and to thereby improve habitat quality (e.g., Platts 1979; Brookes 1985; Newbury and Gaboury 1993). Similarly, erosive channels are sometimes stabilized to encourage the development of a meandering rather than a braided pattern (Newbury and Gaboury 1993).

Table 2. Reach characteristics of weighted useable area (WUA) for fry and parr based on Morantz et al. (1987) criteria.

	PUA_{fry} (%)		AUWfry (m)		PUAparr (%)		AUW_{parr} (m)	
Reach	*Q*1	*Q*2	*Q*1	*Q*2	*Q*1	*Q*2	*Q*1	*Q*2
1	16	27	6	9	16	24	6	8
2	16	33	6	1	11	20	4	6
3	16	41	11	21	15	25	11	12
4	13		4		12		3	
5	14	15	4	4	11	16	3	4
Mean	16	29	7	11	13	21	6	8

Note: Percent useable area (PUA) is WUA divided by the total wetted reach area; average useable width (AUW) is WUA divided by the reach length. *Q*1 and *Q*2 refer to early summer and late summer flows, respectively (mean discharge at *Q*1 = 24 m^3/s; mean discharge at *Q*2 = 8 m^3/s). Means exclude reach 4.

Our data shows that alluvial channel form, on its own, is a primary determinant of habitat availability. On the lower Nouvelle River, planform variations between study reaches with similar formative discharges and daily flows led to as much as a fivefold difference in AUW for fry and threefold for parr (Table 2). Conversely, in reaches with a high degree of planform similarity (reaches 4 and 5), habitat statistics and depth–velocity distributions were remarkably similar (Fig. 3, Table 2). Our evidence also confirms the findings of Mosley (1982) that unstable river sections can confer some habitat advantages by increasing the supply of hydraulically suitable rearing areas for juvenile salmonids. However, of the three unstable, split-channel reaches in our study, only reach 3 with its large, midstream accumulation of gravel and cobble scored high in WUA. Double-channel reaches, 4 and 5, were too deeply incised to provide a large quantity of usable habitat. Clearly, hydraulic habitat depends strongly on the size of the midchannel bars and their degree of incision, rather than simply on the number of thalwegs or separate channels. Our study also provided some insight into the influence of channel curvature on habitat suitability. Although channel curves are generally considered desirable in fish-bearing streams (e.g., Newbury and Gaboury 1993), our data show that they do not always ensure habitat availability. Deeply incised pools and an obscured pool–riffle sequence are often associated with extremely tight curves (Jackson 1975). Such channel incision was observed in the highly curved reaches 4 and 5 of our study, resulting in poor habitat scores. Finally, stage plays an important role in determining the habitat values associated with a given planform type. For example, in an extremely dry summer, reach 3 could potentially become too shallow for parr, and the deeper, moderately meandering reaches 1 and 2 would rank highest in suitable habitat.

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Table 3. Differences in sand content in substrate samples spanning bank erosion sites.

Bank	Migration rate (m/year)[a]	Bank material input (t/year)		Fine content (% <2 mm)			Sample location (Fig. 2)
		Total input[b]	Fines (<2 mm) input[c]	Upstream	Downstream	Difference	
A	5.4	3180	960	12.7	17.3	4.6	4
A				11.3	8.4	–2.9	2
B.1	3.6	500	220	7.6	6.8	–0.8	2
B.1				8.2	3.3	–4.9	3
B.2	3.6	1400	560	22.8	18.6	–4.2	1
B.2				6.8	9.9	3.1	2
C	4.5	1220	430	15.0	24.2	9.2	1
C				12.3	7.6	–4.7	2
C				6.5	3.2	–3.3	3
C				9.7	13.0	3.3	4
D.1	3.3	630	320	8.5	5.3	–3.2	3
D.2	10.5	2900	1310	4.8	10.2	5.4	3
E.1	4.5	1220	730	11.0	6.8	–4.2	2
E.2	4.5	540	270	6.8	5.3	–1.5	2
E.2				17.5	8.0	–9.5	4

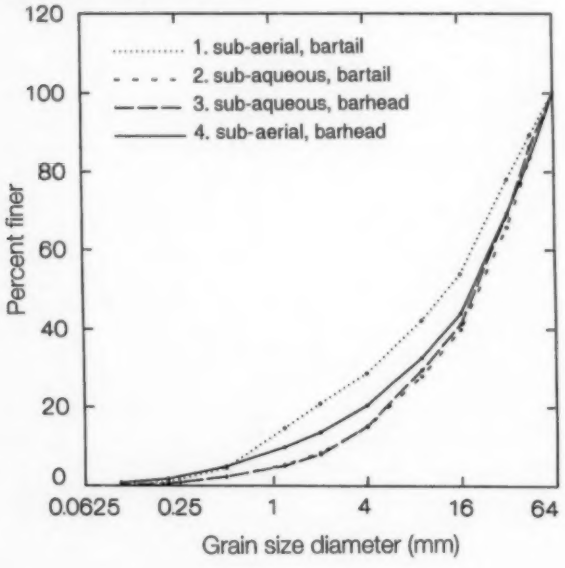
Note: Samples upstream and downstream of each bank site were paired according to lateral locations on riffles (Fig. 2). Positive differences in column 7 indicate enrichment by fines downstream from eroding banks.

[a] Migration rate at the point of maximum bank retreat, measured from 1981 and 1992 aerial photos.

[b] Total input is the product of the average bank migration rate, the average bank height from the thalweg, and the total length of the eroding cutbank.

[c] Column 3 (total input) times the mean percent fines content of bank materials.

Fig. 4. Composite grain-size curves for all riffle samples by generic sample location (defined in Fig. 2).



Weighted useable area results from this study should be viewed with some caution. Several authors have questioned the transferability of preference curves between rivers, and indeed, our choice of curves is not ideal because the largest streams in the Morantz et al. (1987) data set are somewhat smaller in mean annual discharge (11.2 m^3/s) than the Nouvelle River (26 m^3/s). Note, however, that the Morantz et al. curves, used here, are consistent with those developed by Heggenes (1990) for Atlantic salmon in Norwegian streams. Unfortunately, preference curves for the Nouvelle River and similar streams in the region are not available. Nonetheless, the depth–velocity distributions in Fig. 3 accurately reflect the hydraulic habitat contrasts between the reach types. We believe that our data capture hydraulic habitat variations among reaches reliably and that the WUA calculations based on these data are valid for the comparative purposes of this study. It is clear from Fig. 3 that planform has a great potential to influence channel hydraulic conditions and that spatial variations in channel morphology must be taken into account in the evaluation of river habitats, especially at low stage. In wandering systems such as the Nouvelle River, adequate descriptions of habitat availability thus require a habitat sample reflecting the large variety of planform types.

In our study into the effect of bank erosion on spawning gravels, significant fines enrichment in riffle gravels located downstream of rapidly retreating sandy cutbanks could not be detected. If enrichment did occur, it appeared to be relatively weak and hard to detect given the inherently large spatial variability in the size composition of gravel substrates. In the summer period prior to spawning, riffles maintained a grain-size composition that met published criteria for spawning suitability, even after large spring-time inputs of fine sediments from cutbanks.

The relative dearth of riffle fines found in unstable sections of the Nouvelle River can be attributed to a variety of fluvial mechanisms. At high flows, secondary currents induced by channel curvature tend to transport fines released by bank erosion to upper elevations on point bars. Also, hydraulic forces clean fines from the upstream end of bars, whereas in "bartail" areas, fines accumulate (Parker and Andrews 1985; Dietrich 1987). Bed topography and related variations in shear stress also help to sort bed materials. At low flows, fines are

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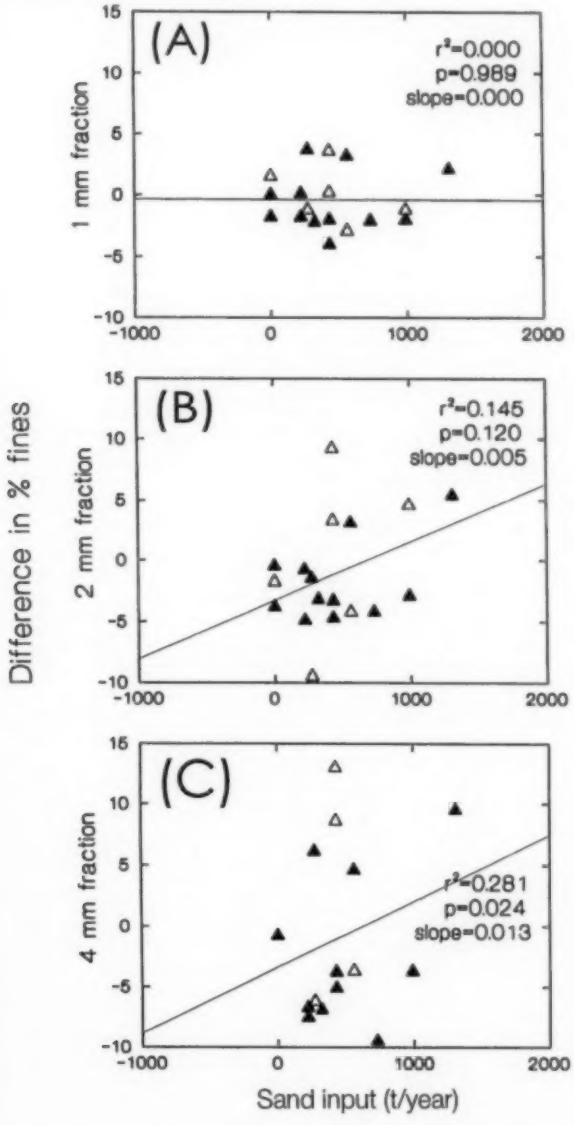
Fig. 5. Differences in the percent fine content of riffle substrate between locations upstream and downstream from eroding cutbanks plotted against yearly cutbank sand input. Various fines fractions are shown: (A) <1 mm, (B) <2 mm, and (C) <4 mm. Solid triangles represent differences between sub-aqueous samples; open triangles represent sub-aerial differences. Positive ordinates denote downstream enrichment by fines.



winnowed out of areas of high shear stress, such as riffles, towards bed units with low shear stress, such as pools, channel margins, and backwaters (Lisle 1989; Lisle and Hilton 1992; Lisle and Madej 1992). Overall, in the moderately powerful Nouvelle River (70–100 W/m^2 of formative flow power over a 4- to 5-cm cobble substrate) hydraulic sorting mechanisms maintain good spawning substrate even where the inputs of cutbank fines are high. This finding agrees with Lisle (1989) who showed that the occurrence of fining in a river depends not only on the volume of fines entering the channel but also on the hydraulic power that regular high flows exert. Habitat managers should assume, therefore, neither that fine sediment input from bank erosion and other sources always degrades spawning habitat nor that rapidly eroding cut banks necessarily require stabilization.

Table 4. Paired *t*-test results for the effect of eroding cutbanks on the fines content of riffles.

Trial	Size parameter	Mean difference (%)	*N*	SD	*P*
A	% <1 mm	0.4	15	2.5	0.510
	% <2 mm	0.9	15	5.0	0.492
	% <4 mm	–0.2	15	8.3	0.904
	% <16 mm	3.0	15	2.2	0.356
	D_{50}	–1.3	15	7.4	0.516
B	% <1 mm	0.2	8	2.6	0.809
	% <2 mm	–1.3	8	3.6	0.348
	% <4 mm	–0.8	8	6.8	0.761
	% <16 mm	0.0	8	13.7	0.996
	D_{50}	3.7	8	7.0	0.178

Note: Paired upstream and downstream samples from the same generic sample location are compared in Trial A. In Trial B, upstream–downstream pairs of sub-aqueous samples (locations 2 and 3 pooled) are compared. *N*, total number of pairs; SD, standard deviation of the difference; *P*, probability of zero enrichment effect. For the 1- to 16-mm fraction, positive values in column 3 (mean difference) are associated with downstream enrichment by fines. For D_{50}, enrichment is indicated by a negative value.

Other questions relating bank erosion and spawning habitat quality remain, however. Large inputs of gravel from eroding banks may induce greater depths of scour and fill during high flow events and could deeply bury or scour redds during the egg incubation period. Longer term studies of gravel dynamics during the period between spawning and emergence are required to evaluate the full impact of lateral instability on the availability of Atlantic salmon spawning habitat in the Nouvelle River and elsewhere.

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Accuracy of diatom-inferred total phosphorus concentrations and the accelerated eutrophication of a lake due to reduced flushing and increased internal loading

Brian Rippey, N. John Anderson, and Robert H. Foy

Abstract: The sedimentary record in a small eutrophic lake (White Lough, Northern Ireland) is used to check the accuracy of reconstructing lake total phosphorus concentrations using diatoms and to propose a mechanism for the accelerated eutrophication of certain lakes. The diatom-inferred total phosphorus and sedimentary calcium, magnesium, potassium, iron, manganese, and phosphorus concentrations were measured in a dated sediment core and compared with lake monitoring results for 1978–1983. The values of and trends in diatom-inferred total phosphorus concentration closely follow the monitored concentrations. There was a period of accelerated eutrophication between 1973 and 1979 and the evidence indicates that this was due to reduced hydraulic flushing of the lake during a dry period and a sudden increase in the sediment phosphorus release rate. The results indicate that meso- to eutrophic lakes with hydraulic residence time around a year may be susceptible to this type of accelerated eutrophication.

Résumé : Nous avons utilisé le profil sédimentaire d'un petit lac eutrophe (White Lough en Irlande du Nord) pour vérifier la précision de la reconstruction des concentrations de phosphore total dans le lac au moyen de diatomées et pour suggérer un mécanisme expliquant l'eutrophisation accélérée de certains lacs. Les concentrations totales de phosphore, déduites au moyen des diatomées, et les concentrations de calcium, de magnésium, de potassium, de fer, de manganèse et de phosphore dans les sédiments ont été mesurées dans une carotte datée, et comparées aux résultats obtenus par échantillonnage dans le lac entre 1978–1983. Les valeurs et les tendances de la concentration de phosphore total calculées à partir des diatomées sont conformes aux concentrations mesurées. On a noté une période d'eutrophisation accélérée, entre 1973 et 1979, qui serait liée à une diminution de l'effet de chasse d'eau du lac au cours d'une période de sécheresse et à un accroissement subit du taux de libération du phosphore par les sédiments. Les résultats révèlent que les lacs eutrophes à mésotrophes dont le temps de séjour de l'eau est d'environ un an sont sensibles à ce type d'eutrophisation accélérée.
[Traduit par la Rédaction]

Introduction

Eutrophication of freshwaters has long been a problem, but the recent rise in the diffuse export of phosphorus from land (Krug 1993; Sharpley and Withers 1994; Foy et al. 1995) makes it now more important. While there is a solid scientific understanding and basis for managing the eutrophication of freshwaters (OECD 1982; Vollenweider 1987; Rast and Holland 1988), improvements are still necessary. The sedimentary record has been widely used to describe changes in the trophic state of lakes to provide a basis for management and for historical monitoring. Both the chemical (Williams et al. 1976; Rippey et al. 1982; Engstrom and Swain 1986; Johnson and Nicholls 1989; Edmonson 1991; Anderson and Rippey 1994) and diatom (Bengtsson and Persson 1978; Anderson and Rippey 1994) records have been used to describe the history of phosphorus loading and the change in trophic state of a lake.

An important recent addition to environmental reconstruction using lake sediments is the ability to use diatoms to infer past lake total phosphorus (TP) concentrations (Hall and Smol 1992; Anderson et al. 1993; Bennion 1994). There have, however, been few checks on the accuracy of diatom-inferred total phosphorus (DI-TP) reconstruction using lake sediments (Bennion et al. 1995), and this was the first aim of this study. White Lough (Northern Ireland) was monitored intensively from 1978 to 1983 (Foy 1985), and so the DI-TP concentrations in a high-resolution sediment core taken in 1992 were compared with this historical monitoring information. The results from the White Lough sediment core also showed that there was a period of rapid eutrophication between 1973 and 1979, and so the second aim of this study was to determine the cause of the eutrophication.

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Materials and methods

Study area

White Lough is a small kettle lake located within the main drumlin

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belt of Northern Ireland. The lake area is 7.4 ha, mean depth is 6.2 m, and maximum depth is 10.7 m. It is thermally stratified from late April – early May to September–October, and an anoxic hypolimnion leads to release of phosphorus, manganese, and iron from the lake sediment. Further details are given in Foy (1985) and Foy and Fitzsimons (1987).

The catchment of White Lough is almost entirely devoted to grassland agriculture. It is divided among a number of farmers, but only two farms are actually located within the catchment, the larger of which accounted for over 50% of the catchment up to 1985. Detailed surveys of land use have not been carried out and the following qualitative assessment of land use on the larger of the two farms is based on an interview with the farmer. This farm had an expanding dairy cow herd during the 1960s, but this was sold in 1973, with a switch to beef production, which required a lower intensity of agricultural inputs. Around 1977, the farm was sold and the land rented by five farmers, who used it for summer grazing with no overwintering stock, a further reduction in agricultural intensity. Since 1985, a limited number of cattle have overwintered on this farm but still substantially fewer than prior to 1973. Animal slurry was not spread directly on the land but used as farmyard manure up to 1977. From 1977 to 1984, no animal waste for land spreading was generated due to the absence of overwintering stock. In 1983, approximately 15% of the catchment was field drained, ploughed, and reseeded with grass. From approximately 1988 onwards, another farmer has been enlarging, draining, and reseeding fields, although the area impacted has been less than 10% of the catchment. In summary, the catchment is unusual, as there has been a diminution in farming intensity since 1973, with perhaps a limited recovery since 1988 and little use of animal slurries.

Methods

A 20-cm-long sediment core (WHI 5) was taken in May 1992 using a modified Kajak–Brinkhurst corer (Brinkhurst 1974) from the deepest part of the lake at approximately 10 m depth. The core was sealed in the field and transported vertically 40 km to the laboratory for subsampling where it was extruded at 1-cm intervals and the dry weight and loss-on-ignition measured using standard methods (Dean 1974). The core was dated using gamma spectroscopy counting methods (Appleby et al. 1986) that permit simultaneous determination of ^{210}Pb, ^{226}Ra, ^{137}Cs, ^{134}Cs, and ^{241}Am.

Sample preparation for diatom counting followed standard methods (Renberg 1990). Around 350 valves were counted for each level and the results expressed as a percentage of total count. Taxonomy used standard floras (see Battarbee 1986 for a list), except for some of the small centric diatoms which have undergone revision recently (Anderson 1990).

The eplimnetic DI-TP was estimated using weighted averaging regression and calibration, implemented in the program WACALIB 2.1 (Line and Birks 1990). The training set used in the calculation for this study is based on 49 Northern Irish lakes that cover a TP range of 15–800 $\mu g \cdot L^{-1}$. The ability of the training set to predict phosphorus is good; the $RMSE_{boot}$ (i.e., independently validated using bootstrapping techniques) of the log predicted – log observed plot is 0.244 ($r^2 = 0.73$). The technical details of the weighted averaging transfer function method are given in ter Braak and Prentice (1988) and Birks et al. (1990), and more specific details of the Northern Irish data set can be found in Anderson et al. (1993) and Anderson and Rippey (1994). All DI-TP values were back-transformed from log values.

Chemical analyses were undertaken on dried subsamples. The samples were ground using a mortar and pestle, dried at 105°C, and approximately 0.3 g treated sequentially with hydrofluoric, nitric, and perchloric acids (Bock 1979). The residue was taken up in hydrochloric acid and made up to 25 mL volume. All the acids were ARISTAR quality. The metals were determined by flame atomic absorption spectrometry (Perkin-Elmer 2380), with 50-fold dilution (Hamilton Microlab 1000) by 0.1% lanthanum chloride for the major cations. Phosphorus was determined by solution spectrometry (Murphy and Riley 1962) after 20-fold dilution. No replicates were included with this core, but the precision of results (standard deviation) from sediment analysed in this laboratory are is follows (see also Rippey et al. 1982): 0.12–0.30 mg $Mg \cdot g^{-1}$, 0.34–0.54 mg $K \cdot g^{-1}$, 0.34–0.42 mg $Ca \cdot g^{-1}$, 0.11–0.13 mg $P \cdot g^{-1}$, 0.24–0.43 mg $Fe \cdot g^{-1}$, and 0.011–0.017 mg $Mn \cdot g^{-1}$.

Lake TP concentrations from July 1978 to November 1982 have been previously presented (Foy 1985) and were based on fortnightly samples of a 0- to 4-m composite and every metre from 5 to 10 m depth. The present study draws on additional samples taken at the same frequency up October 1983, with two samples in May 1977, and four samples taken during 1991 and 1992. Loadings of iron and TP between 1978 and 1982, presented by Foy (1985), were based on sampling the four inflows to the lake. Revised TP and iron loadings are presented here for November 1978 – October 1983 along with new loadings for manganese. Total manganese was measured by flame atomic absorption spectrometry after acidification. The iron loading was enhanced by 774 kg Fe with the addition of ferric aluminium sulphate (FAS) in February 1980. The White Lough catchment is not flow gauged, and flows were estimated by daily runoff from the River Blackwater catchment, of which White Lough forms a small portion. A mean flow-weighted concentration was calculated for the entire period of sampling which was then multiplied by runoff to produce a loading (Foy 1985). Therefore, variation in annual loadings arises from variation in annual flows, as the same flow-weighted concentration was used for each of the years. Because the sampling periods were longer than those used by (Foy 1985), the flow-weighted concentrations of iron and TP differ slightly: TP is 5.1% higher and iron 3.8% higher. The annual hydraulic residence times for White Lough from 1960 to 1990 are based on Blackwater flows between 1971 and 1990 and on runoff values from the adjacent Erne catchment from 1960 to 1971.

Results

Sediment chronology

The ^{210}Pb and unsupported ^{210}Pb concentrations are almost constant over the length of the sediment core, and this makes dating by this method alone difficult (Fig. 1). Calculations with the CIC ^{210}Pb dating model (Appleby and Oldfield 1978) suggest that the entire core spans 20–30 years. However, there are clear ^{137}Cs, ^{134}Cs, and ^{241}Am profiles and these can be used, along with ^{210}Pb, to derive a chronology. The ^{137}Cs activity– depth profile has two distinct peaks. The upper peak at 5.5 cm coincides with a peak in ^{134}Cs activity and records fallout from the Chernobyl incident in 1986. The deeper ^{137}Cs peak at 18.5 cm, which coincides with a peak in ^{241}Am, records the period of maximum fallout from nuclear weapons in 1963 (Appleby et al. 1991). A CRS model ^{210}Pb chronology for the core was calculated (Fig. 1) using the ^{137}Cs peak at 18.5 cm as a dated (1963) reference level (Oldfield and Appleby 1984). There is good agreement between the ^{210}Pb date for 1986 (5 cm) and the independently derived ^{134}Cs date for the Chernobyl incident (5.5 cm). The dating shows that the dry mass accumulation rate increases steadily up the core by a factor of 2 from around 0.03 to 0.06 $g \cdot cm^{-2} \cdot year^{-1}$.

An assessment of the accuracy of the sediment chronology is as follows. The CRS model produces a standard error for all dates of ±2 years, but it is necessary to take into account any random factors and bias due to sediment mixing. The lake area (7.4 ha) to mean depth (6.2 m) ratio indicates that intermittent complete mixing is expected in White Lough, especially at

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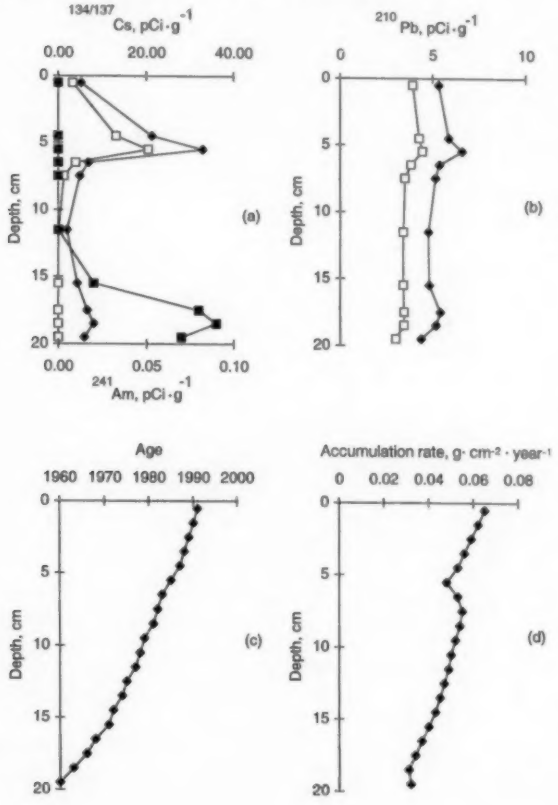
Fig. 1. Variation of (*a*) ^{134}Cs (□), ^{137}Cs (◆), and ^{241}Am (■) activities, (*b*) total (◆) and unsupported (□) ^{210}Pb activities, (*c*) age, and (*d*) sediment accumulation rate with depth in White Lough sediment core WHI 5. 1 pCi = 37 mBq.



autumn overturn (Hilton 1985). A framework for assessing sediment mixing, which uses a dimensionless parameter *G* to describe the importance of mixing, was provided by Guinasso and Schink (1975). The skewness (–1.85) of the ^{134}Cs profile implies a *G* value of 0.7, and this, along with the standard deviation (1.5 cm) of the ^{134}Cs profile and linear sediment accumulation rate above 7.5 cm (1 cm·year^{-1}; Fig. 1), allows a sediment mixing coefficient of 1.1 cm^2·year^{-1} and mixed layer depth of 1.9 cm to be estimated. This sediment mixing coefficient is within the range for lakes, although at the lower end. Sediment mixing displaces sediment downwards, and a *G* of 0.7 implies that this displacement is about 0.6 of the mixed layer depth, i.e., 1.1 cm (Guinasso and Schink 1975). This would give a bias in the sediment chronology relative to the actual date of a pulse input of about 1 year (Fig. 1). This assessment is based on the skewness of only six points on the ^{134}Cs–depth profile, and so the estimates of mixing rate and depth are imprecise. It is also possible that the skewness is due to redistribution of ^{134}Cs and ^{137}Cs initially deposited in shallow parts of the lake to deeper areas as a result of winnowing (Anderson et al. 1987). Nevertheless, our assessment of sediment mixing indicates that any bias due to this cause is about 1 year, which is within the random error of the CRS model ^{210}Pb chronology of ±2 years.

Sediment accumulation rate and basic constitution

As the magnesium (mean 4.86 mg·g^{-1}, SD = 0.33) and potassium (mean 13.71 mg·g^{-1}, SD = 0.96) concentrations are almost constant (see Fig. 4*a*), there are no changes in the composition and proportions of silicate minerals in the sediment over the time period covered by the core (Mackereth 1966; Engstrom and Wright 1984). Although the sediment accumulation rate increases by 2 (Fig. 1), overall, the magnesium, potassium, and sediment accumulation rate results demonstrate a stable sedimentary environment over the time period represented by the core.

DI-TP

The sedimentary diatom assemblages at White Lough were dominated by planktonic diatoms, with only a few benthic taxa having continuous profiles (e.g., *Achnanthes minutissima*, *Cocconeis placentula*) (Fig. 2). The basal sediments (below 18 cm) were dominated by *Aulacolseira subarctica* (at values >20%) and *Aulacolseira ambigua*. There were a number of other planktonic taxa towards the base of the core, but mainly at values <10% (e.g., *Fragilaria crotonensis*, *Stephanodiscus neoastraea*, *Cyclotella radiosa* agg., *Aulacolseira islandica*, *Asterionella formosa*). From 18 cm depth, *Cyclostephanos dubius* increased to an abundance over 40% at 16 cm, but then declined again. The central part of the core (between 9 and 13 cm) was dominated by *Stephanodiscus hantzschii* and *Asterionella formosa*. *Stephanodiscus hantzschii* was present at >10% from 18 cm but increased rapidly from these low values to reach approximately 60% at 11.5 cm. *Aulacolseira islandica* values also increased slightly. A number of taxa present in the earlier zone were either reduced dramatically in abundance (*Aulacolseira subarctica*) or were absent (e.g., *Cyclotella radiosa*). In approximately the upper 7 cm (1980–1990), *Stephanodiscus hantszchii* declined precipitously. *Asterionella formosa* was also reduced, while there was a reciprocal increase in *Aulacolseira subarctica*. A number of other taxa also returned above 8–9 cm (e.g., *Cyclotella radiosa*, *Stepahodiscus alpinus*) or were present as subdominants for the first time (e.g., *Synedra acus*, *Diatom elongatum*), while *Cyclotella ocellata* reached approximately 10% at around 5 cm. This diatom stratigraphy, notably the expansion of *Stephanodiscus hantzschii* in the late 1970s, represents a rapid eutrophication of the lake followed by a recovery. There is considerable species turnover within a short time period, presumably as a result of the eutrophication of the lake. The DI-TP profile provides a concise summary of this eutrophication (Fig. 3).

The DI-TP concentrations are compared with the annual mean, maximum, and minimum TP concentrations in Fig. 3*a*. When making this comparison between the results from the sediment core and the monitoring results, the error in the sediment chronology of ±2 years must be noted. All the annual mean lake TP values fall within the range DI-TP ± 1 SD, and the main trends in lake concentration are recorded by the DI-TP. In particular, the sharp post-1977 decline in DI-TP parallels a similar decline in lake TP from 1978 to 1980, and the slight increase in DI-TP after 1980 is also evident in the lake TP, although the DI-TP values were consistently higher than the corresponding lake concentrations after 1980, with differences between 20 and 30 µg·L^{-1}. To check if the DI-TP values correspond more closely to the phosphorus concentrations at

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Fig. 2. Relative frequency diatom stratigraphy for White Lough sediment core WHI 5. Only dominant taxa are shown.

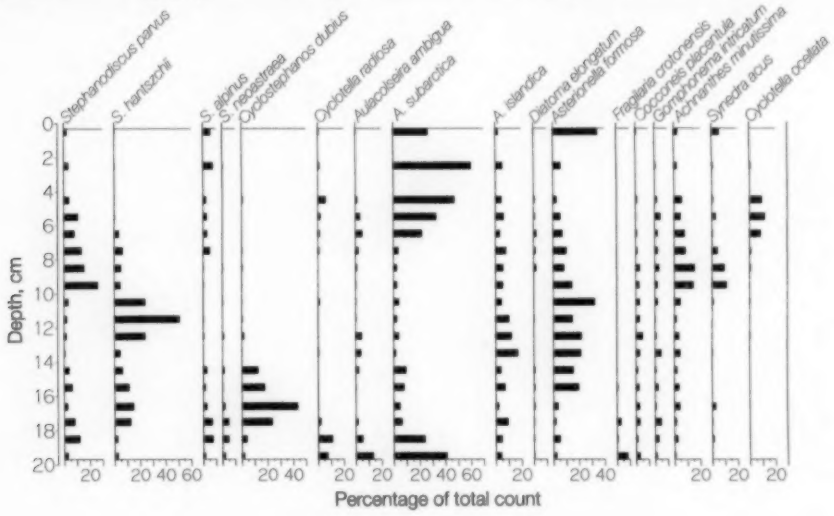
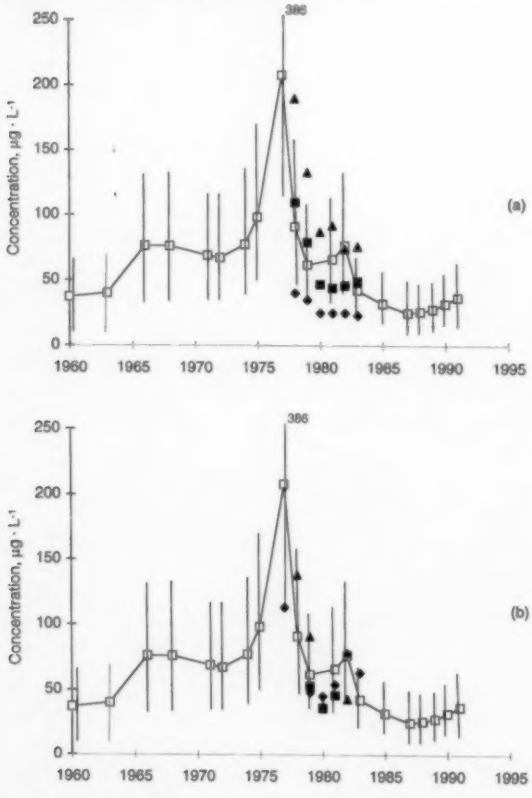


Fig. 3. Variation of DI-TP concentration (□) in White Lough sediment core WHI 5 and the measured (*a*) annual mean (■), minimum (◆), and maximum (▲) and (*b*) October–March mean (▲), April–September mean (■), and May mean (◆) lake TP concentrations. The error bars for DI-TP values are 1 SD and the chronology for the core is given in Fig. 1.



particular times of the year, monthly mean epilimnetic TP values were calculated and, from these, winter (October–March) and summer (April–September) averages produced (Fig. 3*b*). The agreement between DI-TP values and summer or winter epilimnetic TP concentrations is no better than with annual mean values; thus, the diatom model is insensitive to these seasonal variations of lake phosphorus concentrations. The single May 1977 lake TP concentration (113 $\mu g \cdot L^{-1}$) was higher than any subsequent May values and gives further support to the maximum DI-TP value of 208 $\mu g \cdot L^{-1}$ in 1977 (Fig. 3*b*). At the core surface (1992), there is close agreement between the DI-TP of 37 $\mu g \cdot L^{-1}$ (Fig. 3*b*) and the mean value of 36 $\mu g \cdot L^{-1}$ for the four samples taken in 1991 and 1992.

Eutrophication episode, 1973–1979

The DI-TP results (Fig. 3) describe the changes in lake trophic state and show that lake TP concentrations rose from around 40 $\mu g \cdot L^{-1}$ during the early 1960s (18.5–19.5 cm; Fig. 1) to around 70 $\mu g \cdot L^{-1}$ between 1966 and 1972 (14.5–17.5 cm). This was followed by a rapid eutrophication episode between 1973 and 1979 (9.5–13.5 cm) when the DI-TP concentration rose and fell rapidly with a maximum of 208 $\mu g \cdot L^{-1}$. After a secondary peak in 1982 (7.5 cm), the DI-TP fell to values around 30 $\mu g \cdot L^{-1}$ from 1985 (5.5 cm) onward.

The sedimentary calcium concentration was relatively constant, as with the other cations (Fig. 4*a*), except for the period between 1972 and 1979 (9.5–14.5 cm). The maximum calcium and DI-TP concentrations coincide and suggest that an increase in epilimnetic productivity caused the higher calcium concentrations through the formation of calcite. There are few studies that directly show that higher lake productivity causes higher sedimentary calcium carbonate concentrations (Otsuki and Wetzel 1974; Kelts and Ksü 1978), but the field evidence for this is very strong (Kelts and Ksü 1978; Behbehani et al. 1986; Engstrom and Swain 1986; Schelske et al. 1988; Schneider et al. 1990). Provided there are no changes in the calcite sedimentation process as lake productivity varies (e.g., extent of dissolution), then the sedimentary calcium

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profile can be interpreted as an approximate total algal productivity sequence. The sedimentary calcium concentrations drop a little after 1982 (7.5 cm) and stabilise fully at 1985 (5.5 cm).

The DI-TP and calcium profiles provide direct evidence for strong and rapid eutrophication of White Lough between 1973 and 1979, while DI-TP documents an earlier increase in phosphorus concentration from 1963. There is no evidence that the strong eutrophication between 1973 and 1979 was caused by variations in external phosphorus loading. Our assessment of land-use change in the catchment indicates that farming intensity rose during the 1960s and dropped after 1973, with perhaps with a slight rise after 1988. However, it is likely that the earlier change in trophic state was due to an increasing external load, but that the higher algal productivity did not increase the lake pH sufficiently to produce enough quantities of calcite to alter the sedimentary calcium concentrations. Both the DI-TP and sedimentary calcium returned to stable values by 1985.

Eutrophication model

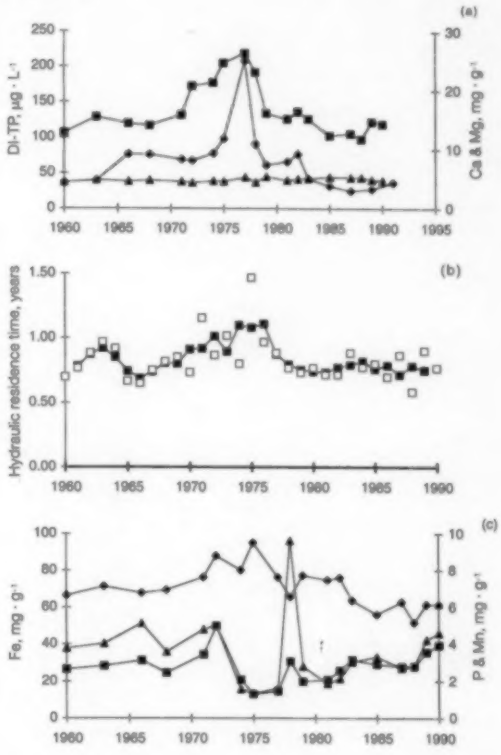
An examination of the DI-TP, calcium, manganese, and TP profiles and consideration of changes in the hydraulic residence time of the lake suggest that the eutrophication episode between 1973 and 1979 was caused by two factors (Fig. 4): reduced flushing of the lake, especially during the autumn–winter period, and an increase in the extent of phosphorus release from deepwater sediments. This explanation can best be presented by using the continuous stirred tank reactor model which is the basis of lake management models (Vollenweider 1975; Ahlgren et al. 1988; Prairie 1989). The steady-state TP concentration is related to the external loading (L), lake mean depth (z), hydraulic flushing coefficient (ρ, fraction of lake volume lost through the outflow each year), and phosphorus sedimentation coefficient (σ, fraction of mean lake phosphorus content permanently lost to sediment each year) as follows:

$$TP = L/z(\rho + \sigma)$$

This representation separates, at least conceptually, the effects of hydraulic flushing from changes in net phosphorus sedimentation. The lake model shows that, in the absence of any changes in lake phosphorus loading, a drop in ρ, which is the same as an increase in lake hydraulic residence time ($\tau = 1/\rho$), causes the steady-state lake concentration to increase. In the same way, a drop in σ, due to an increase in sediment release of phosphorus, causes the lake concentration to rise.

The hydraulic residence time of White Lough varies from year to year, but a simple three-point moving average summarises the general trends (Fig. 4*b*). The main feature was a period of higher residence time between 1971 and 1977, with a maximum in 1975. This period of reduced flushing is dominated by the years 1971, 1973, and 1975, when the residence time was greater than 1 year. The start of the period of higher hydraulic residence time in 1971 was just before the start of the main eutrophication period from 1973 to 1979, within the error of the sediment dating (±2 years), and suggests that reduced lake flushing was involved in the eutrophication episode. However, unless in the unlikely case that there is a strong point source of phosphorus in the inflow, simple variations in steady-state lake concentration caused by changes in flushing cannot be the primary cause of the eutrophication episode. As the TP concentration did not vary with flow in the main

Fig. 4. Variation of (*a*) DI-TP concentration (■) and sedimentary calcium (◆) and magnesium (▲) concentrations, (*b*) annual hydraulic residence time (□) and simple three-point average of annual hydraulic residence time (■), and (*c*) sedimentary iron (◆), phosphorus (▲), and manganese (■) concentrations with time in White Lough sediment core WHI 5. The chronology for the core is given in Fig. 1.



streams in White Lough over the period of monitoring from 1978 to 1983 (mean = 72 $\mu g \cdot L^{-1}$), there is no evidence for a point source of phosphorus in the streams. For the hydraulic residence time to be involved in accelerating eutrophication of White Lough, there also needs to be a source of phosphorus within the lake that can be flushed out to a greater or lesser degree. As about 75% of the water flow through White Lough is between November and March (Foy 1985), washout of nutrients during the autumn–winter period is critical.

The sediment release of phosphorus and manganese during the period of thermal stratification in White Lough is considerable (Table 1) and, further, the proportion of the annual phosphorus and manganese external loads released from the sediment is also substantial (Table 2). When this phosphorus and manganese is redistributed throughout the lake after autumn overturn, it is subject to sedimentation and loss through the outflow during the autumn–winter period (Davison 1993). For the autumn–winter losses from the lake to be considerable, variations in hydraulic residence time from year to year should be around 1 year, as is the case in White Lough (Fig. 4*b*). When the residence time is longer, year-to-year variations are damped, and when shorter, the lake is so rapidly flushed that the lake concentration is determined by the inflow.

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Table 1. May–September net sediment release rates of TP, manganese, and iron in White Lough from 1979 to 1983.

	1979	1980	1981	1982	1983
TP					
Input, kg	7.5	4.3	9.5	3.4	6.2
Export, kg	4.1	1.7	6.6	1.9	4.6
Lake storage change, kg	40.4	3.2	33.0	54.5	35.7
Release, kg	37.0	0.6	30.0	52.9	34.1
Release rate, $mg \cdot m^{-2} \cdot day^{-1}$	5.37	0.09	4.35	7.67	4.94
Release, % of annual input	80.1	1.2	58.8	135.6	68.3
R_{tot}	–0.02	–0.14	0.58	0.74	0.65
Manganese					
Input, kg	9.7	5.3	12.4	4.1	8.0
Export, kg	9.7	1.0	8.5	3.2	4.9
Lake storage change, kg	296.8	118.2	146.6	261.0	455.5
Release, kg	296.8	113.9	142.6	260.0	452.4
Release rate, $mg \cdot m^{-2} \cdot day^{-1}$	43.04	16.52	20.68	37.70	65.60
Release, % of annual input	486	174	211	506	683
R_{tot}	0.41	0.44	0.60	0.82	0.89
Iron					
Input, kg	69.9	40.7	87.4	33.3	58.3
Export, kg	9.0	4.0	10.7	3.3	4.5
Lake storage change, kg	92.7	134.2	178.4	169.2	236.3
Release, kg	31.8	97.5	101.7	139.2	182.5
Release rate, $mg \cdot m^{-2} \cdot day^{-1}$	4.61	14.14	14.75	20.18	26.46
Release, % of annual input	7.6	8.0	22.0	39.1	40.4
R_{tot}	0.68	0.86	0.84	0.88	0.89

Note: Lake storage change is the difference between the mass in the lake at the end of the period and that at the start. R_{tot} (=1 – (annual load out/annual load in)) is the total retention coefficient based on total load in, i.e., external load and load released from the sediment from May to September.

Table 2. TP, manganese, and iron budgets for White Lough by hydrological year from 1979 to 1983.

	1979	1980	1981	1982	1983
TP					
Input, kg	46.2	49.5	51.0	39.0	49.9
Export, kg	84.7	57.3	34.3	24.1	29.2
Lake storage change, kg	–9.9	–69.6	6.5	0.4	10.7
Retention, kg	–28.6	61.8	10.2	14.5	10.0
Areal retention, $mg \cdot cm^{-2} \cdot year^{-1}$	–0.039	0.083	0.014	0.020	0.013
R_{ext}	–0.83	–0.16	0.33	0.38	0.41
Manganese					
Input, kg	61.1	65.6	67.7	51.4	66.2
Export, kg	210.4	100.9	83.5	55.2	55.0
Lake storage change, kg	–71.9	–123.8	–24.6	1.0	6.1
Retention, kg	–77.4	88.5	8.8	–4.8	5.1
Areal retention, $mg \cdot cm^{-2} \cdot year^{-1}$	–0.104	0.119	0.012	–0.006	0.007
R_{ext}	–2.44	–0.54	–0.23	–0.07	0.17
Iron					
Input, kg	416	1225	463	356	452
Export, kg	140	181	91	57	68
Lake storage change, kg	43	–88	12	–32	66
Retention, kg	236	1132	360	331	318
Areal retention, $mg \cdot cm^{-2} \cdot year^{-1}$	0.32	1.53	0.49	0.45	0.3
R_{ext}	0.66	0.85	0.80	0.84	0.85

Note: The 1979 hydrological year runs from 1 October 1978 to 30 September 1979. Lake storage change is the difference between the mass in the lake at the end of the period and that at the start. R_{ext} (=1 – (annual load out/annual load in)) is the retention coefficient based on external load in.

It is also necessary that the phosphorus and manganese that is redistributed after autumn overturn does not sediment rapidly. This is the case for iron and phosphorus in White Lough (Foy 1985), at least before the addition of FAS. Davison (1993) also found that the manganese concentrations decreased slowly over the autumn–winter period in Esthwaite.

The monitoring results show directly that phosphorus and manganese released from the sediment during the stratified period are lost from the lake during the autumn–winter period (Tables 1 and 2). The sedimentary record corroborates and extends this interpretation. The sedimentary phosphorus and manganese concentrations (Fig. 4*c*) increase a little from 1968 to 1972 (14.5–16.5 cm), and our model indicates that this is caused by longer hydraulic residence times (Fig. 4*b*) producing lower losses of these two elements from the lake during the autumn–winter period. The sedimentary phosphorus and manganese concentrations drop sharply between 1972 (14.5 cm) and 1975 (12.5 cm). This drop, at a time when lake productivity was still rising steadily to the peak in 1977 (11.5 cm), as recorded by the calcium and DI-TP concentrations (Fig. 4*a*), must be due to a large increase in the amount of phosphorus and manganese released from the sediment and lost from the lake. An increase in the organic matter flux to the sediment–water interface, as a result of rising lake productivity, causes a decrease in the amount of manganese and phosphorus retained in the sediments. Manganese is directly and phosphorus indirectly sensitive to changes in redox conditions in the hypolimnion and at the sediment–water interface (Davison 1993). This loss from the sediments and from the lake during autumn–winter in White Lough was sufficient to lower the net concentrations in the sediment. Some redistribution to shallower sediments after autumn overturn is also possible. Lakes with anoxic hypolimnia have consistently lower phosphorus sedimentation coefficients (σ) than oxic ones, due to release of phosphorus from the sediments (Nurnberg 1984), and so, according to our model, the steady-state concentration in the lake would rise as anoxia intensified. The sudden increase in the internal loading of phosphorus accelerated the eutrophication of White Lough during the 1973–1979 episode.

Lake recovery

The sedimentary calcium and DI-TP concentrations drop quickly in response to the decline in hydraulic residence time from 1977 to 1979, while the sedimentary phosphorus and manganese concentrations rise (Fig. 4). The calcium and DI-TP concentrations largely stabilised by 1985 and phosphorus and manganese by 1983 (6.5 cm). According to our model, the lower hydraulic residences times resulted in greater losses of manganese and phosphorus during the autumn–winter period and the consequent lower lake productivity lead to a drop in the size of sediment phosphorus and manganese release from the sediment. There was a particularly high sedimentary phosphorus value in 1978 (10.5 cm). This is not an analytical error and was replicated in a further sediment core taken in 1994 (WHI 12). It is unclear if this is due to a particular feature of the redistribution of phosphorus after autumn overturn in 1978 or to addition of the FAS coagulant.

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The monitoring results also suggest that the recovery of White Lough from the eutrophication episode was not immediate. The total retention coefficients, which include the internal loads, are a better measure of changes in lake cycling and indicate that phosphorus stabilised in 1982 and manganese in 1983 (Table 1). That the predicted steady-state phosphorus concentration in White Lough (38 $\mu g \cdot L^{-1}$, OECD 1982; 39 $\mu g \cdot L^{-1}$, Nurnberg 1984) and the phosphorus external retention coefficient (0.54, OECD 1982; 0.57, Nurnberg 1984) are close to the measured values in 1983 (45 $\mu g \cdot L^{-1}$ and 0.41) provides further evidence that the lake was close to steady-state with its external and internal phosphorus loads at that time. The external TP load (0.64 $g \cdot m^{-2} \cdot year^{-1}$) and hydraulic flushing coefficient (1.37 $year^{-1}$) used in these calculations are averages for 1978–1983 (Table 2). Although only a small feature, the DI-TP concentration and sedimentary calcium concentrations rise temporarily around 1982 (7.5 cm) and this coincides, within the error of the sediment chronology (±2 years), with reduced lake flushing in 1983 (Fig. 4). According to the model, the reduced flushing would lead to higher lake phosphorus concentrations, and this, in turn, would raise lake productivity and the higher lake pH would increase calcite production.

FAS addition and iron

Addition of the FAS coagulant in February 1980 produced changes in the behaviour of iron, manganese, and phosphorus in White Lough (Foy 1985). The main changes were lower phosphorus and iron concentrations in the water column during the autumn–winter period and higher iron sediment release rates (Foy 1985). In addition, phosphorus release was completely suppressed in 1980 and there was some reduction in manganese release during 1980 and 1981 (Table 1). However, even with these changes to lake concentrations and sediment release rates, the overall effect of the FAS on the dynamics of iron, manganese, and phosphorus in White Lough during recovery from the eutrophication episode was not large (Tables 1 and 2). The main changes in retention coefficients over the period were a response to recovery from the eutrophication episode, not to the FAS addition. The drop in phosphorus total retention coefficient in 1980 was due to the FAS preventing sediment release during that year (Table 1).

The sedimentary iron concentration does not vary much over the time interval covered by the core (Fig. 4*c*), and the total and external retention coefficients vary much less than with phosphorus and manganese (Tables 1 and 2). There are two small features in the iron results. First, the iron concentration increased in 1971 (15.5 cm) and peaked in 1975 (12.5 cm) before falling. This is the behaviour expected from our model for the retention of a chemical based on lake flushing and sediment release, and there is support for these two requirements. The behaviour of iron concentration and hydraulic residence time between 1973 and 1979 is similar (Fig. 4); the iron concentration in the water column during the autumn–winter period falls (Foy 1985) and the sediment release can be up to 40% of the external load (Table 1). However, as discussed above, the higher sediment release rates from 1980 may have been due to the addition of FAS. Second, the sedimentary iron concentrations are a little higher than expected from 1979 (9.5 cm) to 1982 (7.5 cm). These dates bracket 1980, the year when the FAS was added, and thus this 2-cm layer of slightly higher iron concentration is the sedimentary record of the FAS addition.

Discussion

Sedimentary record

The WHI 5 sediment core from White Lough provides a high-resolution sedimentary sequence covering the period from 1960 to 1992 in 20 cm. Our assessment of the sediment chronology is that any error due to the dating model and sediment mixing is ±2 years. However, the closeness with which actual events coincide with the sedimentary record indicates a very accurate record. In particular, the changes in DI-TP concentration follow closely the monitoring results and the small peak in sedimentary iron concentration dates to the year of the FAS addition. That the DI-TP and sedimentary calcium, phosphorus, and manganese profiles of an earlier undated core (Anderson et al. 1993) are identical to WHI 5 provides further support for a reliable sedimentary record.

Accuracy of DI-TP

Validation of DI-TP records against long-term monitoring results remains a major research goal (Bennion et al. 1995). Although White Lough was only monitored for 5 years, the high sediment accumulation rate has provided sufficient temporal resolution so that the DI-TP results can be compared with the monitored phosphorus concentrations for the period 1978–1983. There is very good agreement between both the values and trends in DI-TP and the monitoring result calculated as annual mean concentrations. The slight upturn in TP between 1981 and 1983 is closely mirrored in the diatom record (Fig. 1), although the diatom model generally overestimates by about 25 $\mu g\ TP \cdot L^{-1}$ during this period. Importantly, the DI-TP values between 1978 and 1983 fell well within the seasonal range of measured epilimnetic TP concentrations. Given the size of the bootstrap errors for the diatom model for the Irish lakes and the variations in lake TP over a year, the agreement between the DI-TP concentrations and the monitoring results is very good.

The main sources of error in diatom-TP models are sediment sampling and representivity of both surface sediment samples in the training sets and the cores used for the reconstruction, variability in water chemistry sampling and seasonal effects, and errors in defining the model coefficients (i.e., species TP optima: see Bennion et al. 1996). Sedimentation variability (within-lake differences of surface sediment samples) has never been systematically addressed as a possible error source in TP models, but Anderson (1997) demonstrated good repeatability of DI-TP values for six cores taken from a range of water depths in a small Irish lake. The analysis of two cores from White Lough demonstrates a near exact agreement in trends of the two cores, but with slight differences of actual DI-TP values. The maximum DI-TP in core WHI 1 was 230 $\mu g\ TP \cdot L^{-1}$ (Anderson et al. 1993) and 208 $\mu g\ TP \cdot L^{-1}$ in WHI 5, the core used in this study.

The main factor that may account for the systematic bias in the DI-TP estimates is probably a bias in the original water chemistry sampling. The diatom model for Northern Ireland uses "annual" water chemistry values based on four to seven water samples taken over 18 months. This annual mean is

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biased towards winter–spring TP values, when epilimnetic TP concentrations tend to be higher, due to lower biological uptake, replenishment from the catchment, and inputs from the hypolimnion following overturn. However, while there is a good agreement between DI-TP and the monitored value for winter 1979 (Table 2), the differences are greater for the other years. There is clearly a need for a careful evaluation of the seasonality in water chemistry in the original diatom models as well as comparisons of long-term monitored data (~50 years).

These results from White Lough indicate that the DI-TP concentrations are sufficiently close to the real values (i.e., well within the seasonal range for a lake) to make diatom-TP models an acceptable approach to reconstructing lake TP concentrations using sediment cores, particularly when the ability to reconstruct concentrations over 100–150 years is included.

Eutrophication model

The record of change in trophic state provided by the DI-TP and sedimentary calcium concentrations shows that, while the phosphorus concentration in the lake started to increase after 1963, there was a period of strong and rapid eutrophication between 1973 and 1979. The evidence indicates that, while the increase in concentration after 1963 was due to a rising external phosphorus load, the external load probably dropped during the 1973–1979 eutrophication episode. The rapid eutrophication episode was initiated by a reduction in hydraulic residence time in 1971 and two factors were involved, reduced flushing and an increase in the release of phosphorus from sediments during summer thermal stratification. In summary, the lake became more eutrophic when the longer hydraulic residence time caused increased retention in the lake of phosphorus released from the sediment. This eutrophication accelerated when the release of phosphorus from the sediment became substantial at the higher trophic states. The DI-TP concentrations, sedimentary iron, manganese, phosphorus, and calcium profiles, lake hydraulic residence time, and the monitoring results from 1979 to 1983 provide strong support for this explanation. The longer hydraulic residence times in White Lough at times during the 1970s were due to lower runoff during widespread dry summers.

Two factors were identified as critical to our eutrophication model: the release of phosphorus from lake sediments and the varying losses of phosphorus from lakes during the autumn–winter period as a result of changing hydraulic residence time. The release of phosphorus from sediments is a significant feature of lakes with anoxic hypolimnia (Nurnberg 1984). As the phosphorus concentrations in the lake (Nurnberg et al. 1986) and the sediment (Nurnberg 1988) increase, so does the amount of phosphorus released from the sediment. Consequently, high internal loads can lead to eutrophic lakes becoming net exporters of phosphorus (Ahlgren 1977, 1980; Larsen et al. 1979), especially when the external load is lowered. The monitoring results show that White Lough was a net exporter during 1978–1979 and 1979–1980 and the sediment core indicates that this started after 1972. The sudden drop in sedimentary phosphorus and manganese concentrations after 1972 shows that sediment release rose rapidly, rather than gradually (Nurnberg 1991). A sudden increase in the internal phosphorus load was also found in the sedimentary record of eutrophication in Augher Lough (Rippey and Anderson 1996). Nurnberg and Dillon (1993) directly showed that the redox cycling of iron can be an important element in the iron budget of some lakes, and the monitoring and our sediment core results show this to be the case in White Lough.

There is less direct evidence for the influence of hydraulic residence time on the loss of chemicals from lakes, especially materials released from the sediment. Year-to-year variations in water column chemical concentration are only expected in lakes with residence times less than a year. When there are year-to-year variations of inflow concentration, water column concentrations are smoothed in long residence time lakes and do not reach steady-state in lakes with a very short residence time. A lake with a hydraulic residence time around a year may then be expected to respond to annual changes in phosphorus load, as is the case with White Lough. We identified that, for the loss of material released from the sediments to be important, it should not sediment rapidly after autumn overturn. Although more information on the behaviour of iron, manganese, and phosphorus during the autumn–winter period is needed, the iron and phosphorus results from White Lough and the manganese results from Esthwaite (Davison 1993) suggest that there is a gradual decrease in concentration over the autumn–winter period.

The concentrations of phosphorus and manganese in White Lough sediments are lowest when the water column concentrations, as indicated by the DI-TP and monitoring results, are at their highest (1973–1979). Hilton et al. (1985) also found that the sedimentary manganese concentrations were lowest when Blelham Tarn was most productive, and Bengtsson and Persson (1978) found that the sedimentary phosphorus and manganese concentrations were lowest when Bergundasjön was extremely oxygen deficient. While Bortleson and Lee (1974) provided little environmental information on the lakes studied, they did find coinciding minima in the phosphorus, manganese, and iron concentrations in the sediments of North Trout, South Trout, and Little St. Germain lakes in Wisconsin, U.S.A.

The time scale of the recovery of iron, manganese, and phosphorus concentrations in White Lough after the 1973–1979 eutrophication episode differed. The return to higher lake flushing during winter 1977–1978 initiated the recovery to less eutrophic conditions, while the total retention of iron stabilised by 1980, phosphorus and manganese by 1982–1983, and the DI-TP and sedimentary calcium, phosphorus, and manganese concentrations by 1983–1985. More than 5 years was needed before the concentrations and internal cycles of phosphorus, manganese, and calcium returned to predisturbance conditions. The drop in sedimentary phosphorus concentrations during the eutrophication episode and recovery thereafter may be an example of the "self purification" of sediments (Nurnberg 1991).

It is likely that reduced flushing is not such an important influence on lake trophic state in oligotrophic lakes. Because there is little release of phosphorus from the sediments of oligotrophic lakes (Nurnberg 1991), variations in hydraulic residence time should have little influence on the proportion of released phosphorous. It was the change of meso- to eutrophic conditions during the early 1960s that made White Lough more susceptible to the influence of lower flushing.

The results and the proposed explanation for the rapid eutrophication suggest that the sediments of certain lakes may

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contain a record of dry climatic periods, as is the case for the dry conditions during the 1970s in the White Lough sediments. Climate warming has caused the increase in hydraulic residence time of three lakes in the Experimental Lakes Area over the last 20 years (Schindler et al. 1996), and while further corroboration of the processes involved in our model is needed, the DI-TP, calcium, phosphorus, and manganese profiles in the sediments of meso- to eutrophic lakes with hydraulic residence times around a year may contain a record of this aspect of climate. Further, the explanation proposed indicates that, in those areas of the world where climatic warming will result in increased lake hydraulic residence times (Hadley Centre 1995), there may be a risk of accelerated eutrophication in meso- to eutrophic lakes, as was the case during the 1970s in White Lough.

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Characterization and application of a quantitative DNA marker that discriminates sex in chinook salmon (*Oncorhynchus tshawytscha*)

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Abstract: A qualitative male-specific DNA marker (OT-24) was amplified by spPCR (single-primer polymerase chain reaction) from chinook salmon (*Oncorhynchus tshawytscha*) DNA along with several non-sex-linked products. The termini of the male-specific product were sequenced, and a pair of PCR primers were constructed for marker-specific PCR amplification. Dual primer PCR (dpPCR), with the marker-specific primers, amplified a product from both males and females. The amount of dpPCR product amplified from males was at least 100-fold greater than that from females. The quantitative difference between males and females was consistent among geographically distinct populations from western U.S. rivers. In addition, DNA sequence analysis indicated that OT-24 was highly conserved among geographically distinct salmon populations. The qualitative spPCR product segregated through several genetic crosses indicating equal sex ratios among progeny. Identification of the male and female juveniles by dpPCR was consistent with the spPCR analysis. There was no tissue specificity observed by spPCR or dpPCR analysis of this marker. A rapid DNA extraction method and dpPCR analysis were used to nonlethally determine sex ratios in wild spring chinook salmon adults, withheld for genetic and behavioral studies, prior to their development of gross sexual differences in their external morphology.

Résumé : Nous avons amplifié un marqueur qualitatif de l'ADN spécifique aux mâles (OT-24) par spPCR (réaction en chaîne de la polymérase à amorce unique) à partir de l'ADN de saumon quinnat (*Oncorhynchus tshawytscha*), ainsi que plusieurs produits non liés au sexe. Les extrémités des produits spécifiques aux mâles ont été séquencées, et une paire d'amorces de la PCR ont été construites pour l'amplification spécifique du marqueur. La PCR à double amorce (dpPCR), avec les amorces spécifiques des marqueurs, a amplifié un produit provenant à la fois des mâles et des femelles. La quantité du produit de la dpPCR amplifiée à partir des mâles était au moins 100 fois plus grande que dans le cas des femelles. La différence quantitative entre les mâles et les femelles était cohérente parmi des populations géographiquement distinctes des cours d'eau de l'ouest des États-Unis. De plus, l'analyse séquentielle de l'ADN a indiqué un niveau élevé de préservation de la région OT-24 parmi des populations géographiquement distinctes de saumons. Le produit qualitatif de la spPCR a été ségrégué au terme de plusieurs croisements génétiques, ce qui indique des rapports des sexes égaux dans la progéniture. L'identification des juvéniles mâles et femelles par la dpPCR concordait avec l'analyse de la spPCR. On n'a observé aucune spécificité des tissus dans l'analyse de ce marqueur par spPCR ou dpPCR. Une méthode rapide d'extraction de l'ADN et l'analyse de la dpPCR ont servi à déterminer de façon non létale les rapports des sexes chez des adultes sauvages de saumon quinnat de printemps, qui ont été retenus pour des études génétiques et comportementales avant l'apparition des différences sexuelles importantes dans la morphologie externe.

Introduction

The genomes of most fish species have morphologically undifferentiated sex chromosomes. Therefore, mechanisms of sex determination have usually been inferred from genetic rather than cytological analysis. The limited number of cases of heterogamety revealed by cytological evidence has shown that teleosts utilize almost every conceivable type of sex chromosome mechanism including: XX/XY, XX/XO, ZW/ZZ, and multiple sex chromosomes (Thorgaard 1977, 1978; Bertollo et al. 1983; Andreata et al. 1993).

Direct cytological evidence for heteromorphic sex chromosomes in salmonids has been reported for only three species, and in all three species, males were the heterogametic sex. The XX/XY type was reported in rainbow trout (*Oncorhynchus mykiss*), by Thorgaard (1977), though not all males in all populations studied appeared to possess this heteromorphism (Thorgaard 1983). Phillips and Ihssen (1985) revealed that an XX/XY mechanism was also operating in lake trout (*Salvelinus namaycush*). The XX/X0 type was reported in both sockeye salmon and kokanee (*Oncorhynchus nerka*) (Thorgaard 1978; Ueda and Ojima 1984).

In the absence of morphologically differentiated sex chromosomes, genetic analysis of sex-linked molecular markers can discern differences in sex chromosomes at the nucleotide level. Segregation patterns of sex-linked markers can be used to infer mechanisms of sex determination in fish species. The presence of distinct sex chromosomes and the location of sex-linked markers can be visualized by hybridization of these markers to intact chromosomes. Hadrys et al. (1992) suggested that sex-linked markers may be easily identified, even in complex and relatively unknown genomes, by PCR amplification using single primers of random sequence composition,

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commonly referred to as RAPDs (randomly amplified polymorphic DNA).

At present, sex-linked DNA markers have been reported in only two salmonid species, chinook salmon (*O. tshawytscha*) and coho salmon (*O. kisutch*). A repetitive DNA marker, isolated by subtractive cloning by Devlin et al. (1991), qualitatively differentiated male chinook from females. An oligonucleotide primer pair, based on the sequence of the marker, amplified a product from chinook males and not females (Devlin et al. 1994). Du et al. (1993), using a primer pair specific to the growth hormone genes, discovered that the primer set also amplified a single copy psuedogene that is contained only in the male genomes of chinook and coho salmon. Both of these markers segregated through genetic crosses from father to son in a manner consistent with an XX/XY mechanism.

We have identified a sex discriminating molecular marker, using spPCR, that qualitatively differentiated chinook salmon males from females. Conversion of this marker to the more sensitive dpPCR resulted in the amplification of a quantitative difference between males and females. This is the first locus reported in salmonids that exhibits a quantitative difference between males and females. This sex-discriminating marker was successfully applied to a field study to determine the sex of returning wild spring chinook adults from nonlethal tissue samples. In addition, a novel method for the rapid extraction of DNA, of suitable quality for PCR amplification, from fish tissues is described.

Materials and methods

Tissue collection and storage

Chinook salmon samples were collected from the following national fish hatchery (NFH) and salmon culture technology center (SCTC) stocks: (*i*) Coleman NFH, Battle Creek, California, fin samples were collected from phenotypically sexed fall chinook at the time of spawning; (*ii*) Quinalt NFH, Quinalt River, Washington, whole fall chinook alevins were collected; (*iii*) Abernathy SCTC, Abernathy Creek, Washington, liver samples were collected from phenotypically sexed fall chinook at the time of spawning and from the juveniles, at the swim-up stage, resulting from 1:1 crosses of these adults; (*iv*) Dworshak NFH, Columbia River, Idaho, fin and opercle samples were collected from fall chinook smolts that were sexed by dissection; (*v*) Carson NFH, Wind River, Washington, kidney samples were collected from phenotypically sexed spring chinook at the time of spawning; and (*vi*) Warm Springs NFH, Deschutes River, Oregon, fin samples were collected from returning wild spring chinook adults. Samples of *Oncorhynchus* species other than chinook were collected from the following stocks: (*i*) amago (*O. masou*), fin samples were collected from sexually mature 1 year olds, National Resource Institute for Aquaculture in Japan; (*ii*) steelhead (*O. mykiss*) liver samples were collected from phenotypically sexed adults at the time of spawning, Dworshak NFH; (*iii*) sockeye (*O. nerka*), liver samples were collected from phenotypically sexed wild adults at the time of spawning, Cedar River, Washington. All tissues were placed immediately in lysis buffer (150 mM EDTA, 50 mM Tris–HCl (pH 8.0), 2% sarkosyl), transported at ambient temperature, and stored at 4°C.

DNA isolation, purification, and manipulation

Total DNA was extracted from fin, liver, and whole body tissues based on a previously published protocol (Rodriguez 1993). A small piece of tissue was placed in two volumes of lysis buffer, incubated at 65°C for 15 min, homogenized, and then incubated at 65°C for another 15 min. The debris was pelleted by centrifugation at 15 800 × *g* for 5 min. The DNA was precipitated from the supernatant (S/N) by the addition of 0.7 volumes of a polyethylene glycol – sodium chloride solution (20% PEG 8000, 2.5 M NaCl), incubated for 5 min at room temperature, and centrifuged at 15 800 × *g* for 5 min. The S/N was discarded and the pellet was resuspended in 0.2 mL TE buffer (10 mM Tris (pH 8.0), 1 mM EDTA (pH 8.0)). Protein and RNA were precipitated by the addition of 0.5 volumes of 7.5 M ammonium acetate (NH_4OAc), incubated on ice for 5 min, and centrifuged at 15 800 × *g* for 5 min. The S/N was transferred and the DNA was precipitated by the addition of NaCl to 0.1 M and two volumes of 95% ethyl alcohol (EtOH) followed by incubation on ice for 5 min. DNA was pelleted at 15 800 × *g* for 5 min, the S/N discarded, and the pellet resuspended in 0.5 mL TE buffer. The DNA solutions were stored at 4°C.

PCR analysis

Polymerase chain reactions (Saiki et al. 1985; Mullis and Faloona 1987) were carried out in 20-μL volumes containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2.5 mM $MgCl_2$, 0.2% Triton X-100, 200 μM each of dATP, dCTP, dGTP, dTTP (Pharmacia), 0.2 units *Taq* DNA polymerase, 500 ng of each oligonucleotide primer, and 0.4–400 ng of salmon DNA. Amplification reactions were carried out in either a Barnstead/Thermolyne Amplitron or Temptronic thermocycler. SpPCR involved 35 cycles of a temperature regime consisting of denaturation at 93°C for 15 s, primer annealing at 48°C for 1 min following a 30-s ramp from 93°C, and synthesis at 72°C for 1.5 min following a 1-min ramp from 48°C. Prior to the initiation of the cycles, the reactions were incubated at 93°C for 2 min. The temperature regime was the same for dual primer PCR (dpPCR) except the ramp times were eliminated and the annealing temperature varied from 64 to 68°C.

The thermocyclers were calibrated with a scanning thermocouple (Cole-Parmer) and adjusted to ensure accuracy and precision of denaturing, annealing, and synthesis temperatures to within ±0.5°C. Electrophoresis of the amplified products was performed for 1.5 h at 12–16 V/cm in 2% agarose (Sambrook et al. 1989). PCR products were stained with ethidium bromide and visualized using 305-nm ultraviolet light.

SpPCR and dpPCR products were isolated on agarose gels and purified by the glass bead method (Gene Clean, Bio101). SpPCR products were cloned into the pT7Blue vector (Novagen) for sequence analysis. Sequence analysis was performed using the chain termination method of Sanger et al. (1977). DpPCR products were sequenced directly after isolation using thermocycler sequencing and the chain termination method performed by the Center for Gene Research at Oregon State University.

The primer used for spPCR was designated p143 (5′-CAGCACTGGTCGTATGGC-3′) and the sequence was arbitrarily derived from the preprogonadotropin releasing factor gene in Atlantic salmon (*Salmo salar*; GenBank accession No. X79711). The dpPCR primers, p551 (5′-GGCTCTCAAGGAAACCGTGCA-3′) and p559 (5′-GGCTTGTTCTGGATTGATTATCCA-3′), were based on sequence analyses of the OT-24 spPCR product. The primers, p709 (5′-TCAGCCCATTGATGGACTTCT-3′) and p710 (5′-AGCAGGTGACTGTCTCAGGAT-3′) were used as an internal control for dpPCR and were based on the 30-kDa heat shock protein (HSP30; GenBank accession No. U19370).

Results

Identification of male-specific marker

SpPCR was performed on 76 fall chinook salmon samples from Abernathy SCTC using an oligonucleotide primer (p143) that was 18 nucleotides in length and produced a consistent DNA fingerprint. This primer amplified a product, 950 base pairs (bp) in length, from male and not female samples and five

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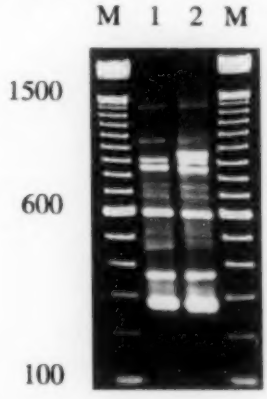
Fig. 1. SpPCR amplification of genomic DNA from female and male samples of chinook salmon. Lanes 1 and 2, female (Ab6) and male (Ab10) samples from the Abernathy NTC, respectively. Product sizes were based on 100-bp DNA size markers located in the lanes denoted by M, with three sizes denoted on the left.



additional monomorphic products ranging in size from 275 to 1000 bp (Fig. 1). To ensure that the male-specific spPCR pattern was not population specific, 24 males and 24 females from Coleman NFH, 2 males and 2 females from Quinalt NFH, and 1 male and 4 females from Dworshak NFH, were analyzed using primer p143. In all cases, the 950-bp product was present in amplified products from male samples but not from female samples.

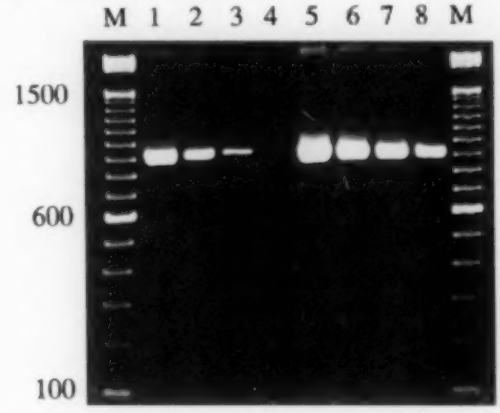
Conversion from spPCR to dpPCR

To screen populations rapidly, the 950-bp spPCR product was cloned for sequence analysis. The cloned product was designated OT-24 and dpPCR primers (p551/p559) were constructed based on terminal sequences. DpPCR analysis of 76 chinook salmon samples (Abernathy SCTC) was performed to determine the fidelity of the primer set p551/p559 in producing the OT-24 marker in males. The OT-24 product was amplified to high concentrations in all males tested. However, females yielded a varied amount of a 950-bp product but of a lesser quantity than males. One female in particular (Ab6) produced a band of an intensity that seemed to approach that typically found in males. To better define the difference in concentration of the amplified product between males and females, it was necessary to evaluate the relationship between DNA template concentration and the resulting amount of amplified product. Serial dilutions of DNA concentrations from the Abernathy female (Ab6) and an Abernathy male (Ab10) were analyzed by dpPCR (Fig. 2). A profile of annealing temperatures (60, 64, 68°C) revealed that 68°C was the optimal annealing temperature to ensure fidelity of primer annealing for dpPCR amplification with p551/p559. At a dilution of 1:1000 there was no further visible band produced from Ab6, and the band in the male was higher in intensity than the female at a 1:10 dilution. This suggested that there was at least a 100-fold difference in the concentration of primer hybridization sites in the genomes of females as compared with males.

Geographic distribution of OT-24

Geographically distinct populations of chinook salmon were

Fig. 2. Marker-specific dpPCR amplification of a genomic DNA dilution series from female and male chinook salmon. Lanes 1–4, dpPCR products amplified from 40 ng, 2 ng, 200 pg, and 20 pg of genomic DNA from female Ab6, respectively; lanes 5–8, dpPCR products amplified from 40 ng, 2 ng, 200 pg, and 20 pg of genomic DNA from male Ab10, respectively. Product sizes were based on 100-bp DNA size markers located in the lanes denoted by M, with three sizes denoted on the left.



then analyzed to see if the observed patterns were consistent over a large geographic range. To reduce the chance of false negatives and to indicate the functional concentration of the DNA, primer set p551/559 was multiplexed with a primer set (p705/706) derived from HSP30. The HSP30 primers amplified a 425-bp product that was monomorphic in chinook salmon and amplified to equivalent levels as OT-24. The following chinook salmon populations were analyzed using equal numbers of males and females: six spring-run individuals from Carson NFH, six late-fall individuals from Coleman NFH (Fig. 3), six fall-run individuals from Quinalt NFH, and eight fall-run individuals from Abernathy NFH. The observed differences in concentration of products between males and females (Fig. 3) were consistent over this broad geographic and run-timing range (data not shown).

Sequence analysis of OT-24

The sequence of an internal 575-bp region of OT-24 was compared in fish from four populations with distinct geographic and run timing differences (Fig. 4). The comparisons were a spring-run male from Carson NFH (Ca8), a late fall run male from Coleman NFH (Co13), a fall-run male from Quinalt NFH (Qn4), and a fall-run male and female from Abernathy NFH (Ab6 and Ab10). The sequence of this fragment was highly conserved among these fish. There was only a single nucleotide difference out of the 575 bp that were compared. Ca8 had a thymine (T) substitution where as the rest of the individuals in the comparison had adenine (A) at the same position.

Genetic analysis

Segregation of the OT-24 marker was observed by performing PCR on 88 progeny samples derived from 4 sets of 1:1 crosses of Abernathy 1995 adults (Ab18-1, Ab18-3, Ab18-5, and Ab18-6). Since the progeny were collected at swim-up and could not be reliably sexed, both spPCR and dpPCR was

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Fig. 3. DpPCR amplification of Coleman samples using the OT-24 and HSP30 specific primers. DpPCR products amplified from three serial dilutions (1:10, 1:100, 1:1000) of genomic DNA from six individuals: lanes 1–3, male Co13; lanes 4–6, female Co44; lanes 7–9, male Co54; lanes 10–12, female Co137; lanes 13–15, female Co157; lanes 16–18, male Co161. Product sizes were based on 100-bp DNA size markers located in the lanes denoted by M, with three sizes denoted on the left.

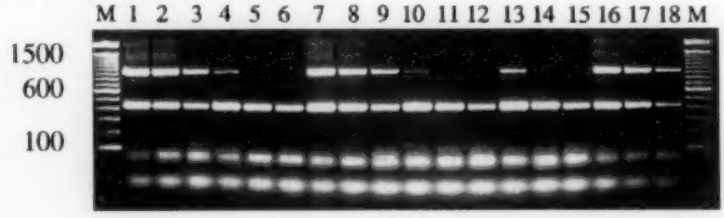

Fig. 4. The consensus sequence of an internal 575-bp region of OT-24 dpPCR products amplified from chinook salmon. The consensus sequence was derived from a comparison of Ca8, Co13, Qn4, Ab6, and Ab10. The arrow indicates the single nucleotide difference identified in this comparison (Ca8, T substitution at position 12).

```
                    ↓
  0 TGATGACAAG GAAGCAGATG AGAGCTTCTT CATTTCCACA ACCGGGAACC
 51 CGATCTACAA CCCATCCAAT AACCTACAAA GGTTTCATTC CAAGTAAGTA
101 CACCACTGTT AAGTAGAACA CTGTTTAATC ATTCCACAAT ACTTATTTGA
151 CAGTACAGTT TTAAGAAAC CAAACTGTTT TTCTTGATCT TTCATCAGAT
201 ACAACTTGCC CAACATCACG AGCCAGGTAG CAAGACGAGT TATGGAGACA
251 AATACCAAAG CCAGCTTCAC AGACCAACAG AAGGCCCTTG TTGCTGACTA
301 CTTGGCCCAT ACAACTGCTA CAGCTGAAAA GCATTACCGC ATGAAGACAC
351 CAGTCAATGC CTGCCTGGGA ATGAAGCTGA TCACCAAAGT TGCCATGTCC
401 GATGAATCTA AGTAAGTATT TACACATATT ATATAAATTA CACTTTACAT
451 TGTCTTGTAA ATGCTGCTAC ATATAGATAC AAATGTGTTG TTATTTTCCC
501 TTTTTCAAGT ATTGAGACTG CAGGCCCCTC CTGTGCAAGC ACAAGTAGAA
551 AGGGAGCATT GTCCAGCTAC AAAAAG
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performed. There was 100% agreement between spPCR and dpPCR results with spPCR positive and negative samples correlating to consistently high and variable low concentrations of the dpPCR product, respectively (data not shown). In all cases the OT-24 marker segregated in a 1:1 manner (male:female molecular phenotypes).

An example of a low female and the range in concentration of her progeny is shown in Fig. 5. These four crosses were chosen because Ab18-3 and Ab18-6 had parental females that produced relatively high concentration of dpPCR product and Ab18-1 and Ab18-5 had parental females that produced relatively low concentrations of dpPCR product. The results indicated that the concentration of the dpPCR product in female parents did not correlate to the concentration of product in progeny that possessed female molecular phenotypes.

Tissue specificity

No qualitative tissue specificity of this marker was apparent in the tissues that were analyzed. Sex was easily discerned by the concentration of PCR product in DNA from fin, liver, kidney, and opercle tissues (data not shown).

An application to conservation biology

This sex-differentiating marker was successfully applied to a field experiment at the Warm Springs NFH where biologists were investigating differences in growth and survival between offspring of wild and hatchery spring chinook reared in natural streams or hatcheries. The wild adults, retained to produce the progeny for the study, needed to be selected within approximately 48 h after entering the hatchery. Normally, the returning adults were sorted each weekday, the hatchery fish were put in a holding pond and the wild fish immediately released upstream.

The study plan required 15 wild females and 11 wild males homozygous at a particular allozyme locus. Adult spring chinook enter the hatchery in May and June, 2–3 months before spawning, and they could not be reliably sexed by their external morphology at that time.

An 8-mm disk of anal fin tissue was removed and cut in two: half for DNA sexing and half for allozyme analysis. The fish were all tagged with individually numbered tail tags. The tissues were analyzed and the results were reported the following day. Only fish with the right genotype and sex were held for the experiment. All others were released upstream.

A rapid DNA extraction method was developed for this study to reduce turnaround time for the results. The samples were boiled in lysis buffer for 20 min, centrifuged at 15 800 × *g* for 15 min, and then a dilution series was made on the supernatant. Differences between males and females were easy to discern at the 1:100 and the 1:1000 dilutions. With this rapid DNA extraction method, sex could be assigned within 6 h after receiving the tissue.

A total of 135 wild fish were analyzed in this study. The 26 fish that were selected and held were not tracked individually, but at the time of spawning the sex ratio proved to be identical to that our prediction: 15 females to 11 males.

Analysis of other salmonids

Amago, steelhead, and sockeye of known sex were analyzed by spPCR (p143) and dpPCR (p551/559) using the OT-24 primer set. SpPCR amplification of all four species produced a banding pattern devoid of a 950-bp or any sex-specific products. DpPCR amplified a 950-bp product from all three species, but it was not sex discriminating.

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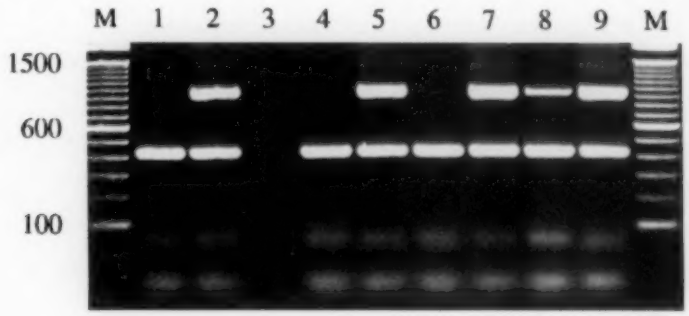
Fig. 5. DpPCR amplification of parental and progeny DNA samples from a genetic cross (Abernathy cross 18-1) using the OT-24 and HSP30 specific primers. DpPCR products amplified from genomic DNA: lane 1, parental female Ab127; lane 2, parental male Ab128; lane 3, blank; lane 4, female progeny 18-1-1; lane 5, male progeny 18-1-2; lane 6, female progeny 18-1-3; lane 7, male progeny 18-1-4; lane 8, female progeny 18-1-9; lane 9, male progeny 18-1-10. Product sizes were based on 100-bp DNA size markers located in the lanes denoted by M, with three sizes denoted on the left.



Discussion

PCR analysis of chinook DNA samples with a single primer (p143) resulted in the amplification of a male-specific product designated OT-24 (Fig. 1). Marker-specific primers (p551/p559) designed to exclusively amplify OT-24 by dpPCR resulted in the amplification of small amounts of OT-24 in female chinook samples (Fig. 2). The consistantly large quantitative difference between males and females amplified with p551/p559 allowed for sex discrimination identical to that observed with p143.

The amplification of a 950-bp fragment with p551/p559 from females, and not with p143, may have been due to greater sensitivity of dpPCR versus spPCR. Alternatively, it is possible that p143 did not have adequate hybridization sites in the female genomes. The difference between males and females in the concentration of their OT-24 dpPCR amplified product could have been due to (*i*) differences in genomic copy numbers, (*ii*) mismatches in the primer hybridization sites, or (*iii*) DNA quality. Since annealing temperature profiles and multiplexing with an internal control eliminated the second and third possibilities, respectively, we conclude that the quantitative differences reflect true differences in copy number.

Although the concentration of the OT-24 product was highly variable in females, copy number in female parentals did not appear to segregate to female progeny (Fig. 5). Since equal copy numbers of OT-24 sequence were not passed from mother to daughter, it is likely that the sequence is not located exclusively on sex chromosomes. It is possible that some or all of the OT-24 product amplified from females and some of the product amplified from males was derived from copies of OT-24 located on autosomes. Greater amounts of amplified products from female progeny than that of their mothers may be due to inheritance of some copies of the sequence through autosomes from their father. Du et al. (1993) reported a similar scenario with amplification of the GH genes. The two functional copies of the gene were present in both female and male chinook; however, the pseudogene copy was only present in males. The inference made was that the two functional copies were located on autosomes, and the pseudogene was located on the Y chromosome. The primer set that amplified the GH genes discriminated each of the three copies of the gene by size, and thus, there were large sequence length differences between the pseudogene and the functional copies.

Sequencing performed on OT-24 products from an Abernathy male chinook salmon and an Abernathy female revealed no nucleotide differences (Fig. 4). This sequence was also highly conserved between males from geographically distant river systems. Conservation of the sex-discriminating OT-24 marker across a large geographic distance and between sexes suggests that it may be of biological importance. A search of GenBank revealed no known sequences having significant similarity.

A 950-bp product was amplified by p551/p559 from DNA samples of male and female amago, sockeye, and steelhead. The concentration of 950-bp product amplified from these three species was not sex discriminating and appeared to be similar to that amplified from chinook males. If the chinook OT-24 sequence is conserved across other salmon species it would support the potential biological significance of this sequence.

In conclusion, the OT-24 marker, regardless of the nature of its sequence, appears to be a practical tool for sex determination in chinook salmon. The OT-24 marker successfully discriminated the sex of 177 spring, fall, and late-fall chinook of known phenotypic sex. There were no discrepancies between phenotype and genotype. DpPCR is extremely reliable and sensitive, so very crude DNA preparations are acceptable. The boiling extraction described here was developed for rapid screening and produces DNA extracts of sufficient quality for dpPCR amplification. Because the OT-24 marker exhibits no tissue specificity, DNA can be extracted from convenient tissues. Only one product is produced, so long electrophoretic runs are not necessary to resolve bands.

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Effects of intermittent hydrogen peroxide exposure on growth and columnaris disease prevention of juvenile rainbow trout (*Oncorhynchus mykiss*)

David J. Speare and Garth J. Arsenault

Abstract: Twice-weekly treatment of juvenile rainbow trout (*Oncorhynchus mykiss*) with hydrogen peroxide at 200 mg·L^{-1} for 1 h was effective in limiting the degree of fin and epithelial damage associated with an experimental thermally promoted outbreak of columnaris disease attributable to *Flexibacter columnaris*. The treatment was at least as effective as a comparable treatment with chloramine-T. The treatment also altered fish growth patterns. During a 7-week growth trial, disease-free treated and untreated fish had similar growth rates, feed consumption, and conversion efficiency. However, during the first 3 weeks, treatment suppressed growth through inhibited conversion efficiency. During the final 4 weeks, treated fish entered a compensatory growth phase marked by significantly increased growth and appetite compared with controls and recovery of conversion efficiency to match controls. Gills from treated fish were no different from control fish at the end of the trial.

Résumé : Le traitement de juvéniles de truite arc-en-ciel (*Oncorhynchus mykiss*) deux fois par semaine avec du peroxyde d'hydrogène à 200 mg·L^{-1} pendant 1 h a réussi à limiter l'ampleur des lésions des nageoires et de l'épithélium associées à une poussée expérimentale de l'infection à *Flexibacter columnaris* favorisée par la température. Le traitement a été au moins aussi efficace qu'un traitement comparable à la chloramine-T. Il a également modifié les schémas de croissance des poissons. Pendant un essai de croissance de 7 semaines, des poissons exempts de la maladie, traités et non traités, présentaient des taux similaires de croissance, de consommation des aliments et d'efficacité de la conversion énergétique. Toutefois, pendant les trois premières semaines, le traitement a annulé la croissance en entravant l'efficacité de la conversion énergétique. Pendant les quatre autres semaines, les poissons sont entrés dans une phase de compensation marquée par une hausse notable de la croissance et de l'appétit par rapport aux poissons témoins, et à une remontée de l'efficacité de la conversion qui les a ramenés au niveau des témoins. À la fin de l'essai, les branchies des poissons traités n'étaient pas différentes de celles des poissons témoins.
[Traduit par la Rédaction]

Introduction

The use of prophylactic chemical treatments for infectious diseases of external mucosal (gill and skin) surfaces of fish is practiced in high-intensity commercial channel catfish (*Ictalurus punctatus*) (Bodensteiner et al. 1993), salmonid (Speare and Ferguson 1989; Bullock et al. 1991; Thorburn and Moccia 1993; Powell et al. 1994; Cipriano et al. 1996), and striped bass (*Morone saxatilis*) (Reardon and Harrell 1990) aquaculture. This reflects the high prevalence of infectious gill and skin diseases within intensive aquaculture, particularly in freshwater systems, the current lack of available vaccines, and the poor surface-protective mucosal immune response in fish as demonstrated by re-occurring infections (Speare and Ferguson 1989; Heo et al. 1990; Urawa 1992; Lumsden et al. 1994).

For salmonids reared in fresh water, the specific diseases that are common, and against which chemoprophylactic regimes are used, include bacterial gill disease (BGD), columnaris disease, protozoal infections, and saprophytic fungi (Daoust and Ferguson 1983; Speare and Ferguson 1989; MacMillan 1991; Urawa 1992; Newbound et al. 1993; Smith et al. 1993; Bullock et al. 1994). Specifically, in parts of Canada, these are the most common "problem" diseases in commercial freshwater salmonid farming (Speare and Ferguson 1989; Thorburn and Moccia 1993; Powell et al. 1994; Speare et al. 1995*a*).

The most common chemicals used for freshwater-reared farmed salmonids are formalin and chloramine-T (Speare and Ferguson 1989; Thorburn and Moccia 1993; Smith et al. 1993). These are both relatively broad spectrum against bacteria and protozoal agents (Robertson 1985; Griffin 1989; Schnick 1988; Wahli et al. 1993; Tojo et al. 1994; Ostland et al.1995; Cipriano et al. 1996). Currently, formalin is licensed for use in North American salmonid aquaculture (Bodensteiner et al. 1993), but chloramine-T is not (Bullock et al.1991; Cipriano et al. 1996). Hydrogen peroxide has been examined as an alternative to chloramine-T (Roberts 1995; Rach et al. 1995), and has recently become popular at high concentrations as a treatment for salmon lice (*Lepeophtheirus salmonis*) in sea cages (Thomassen et al. 1993; Bruno and Raynard 1994). Because of its breakdown to water and oxygen, it

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is considered a low regulatory priority chemical (Armstrong 1994; Rach et al. 1995). However, few studies have addressed the effective dosages or the target-animal safety of this chemical in commercial farm settings (Rach et al. 1995), although it is known to cause severe gill pathology (Johnson et al. 1993) at high doses needed for sea lice control.

The objective of this research was to investigate aspects of the efficacy, safety, and effect on rainbow trout production of a prophylactic treatment regime using hydrogen peroxide. Using a semicommercial production setting, the hypotheses are whether hydrogen peroxide treatment affects (*i*) the degree and incidence of dermatitis due to *Flexibacter columnaris* when compared with chloramine-T treatment, (*ii*) growth and production indices, and (*iii*) gill histopathology.

Materials and methods

Efficacy of hydrogen peroxide and chloramine-T for columnaris control

Procedures used in the study were according to guidelines established by the Canadian Council on Animal Care.

A group of 630 diploid rainbow trout (mean weight 35 g) housed in the aquatic animal facility at the Atlantic Veterinary College (AVC), Charlottetown, P.E.I., was noted to have partially eroded dorsal fins. The severity and prevalence increased gradually over a 2-week period. These fish were in tanks supplied by well water (total hardness 230 mg·L^{-1} (as calcium carbonate), pH 7.7) at 10°C. A diagnosis of localized columnaris disease due to *F. columnaris*, was reached based on the results of the following diagnostic tests applied to 10 severely affected fish. The disease was confirmed by light microscopy of unstained and Gram-stained whole mounts taken from affected fins (Austin and Austin 1987; Noga 1993) and tissue sections of fins, skins, and visceral organs (Ferguson 1989; Speare and Mirsalimi 1992). Additionally, mucous smears and skin scrapings were taken from the areas of fin damage and evaluated with an indirect fluorescent antibody (IFA) method using monoclonal antibodies previously developed to detect *F. columnaris* (Speare et al. 1995*a*).

From this group, 180 affected fish were selected and evenly distributed by random selection into each of six 70-L circular fibreglass tanks. The early infection with *F. columnaris*, initially restricted to the dorsal fin, was encouraged to become active and spread to subjacent skin by increasing the water temperature, since this increases the pathogenicity of this organism (Wakabayashi 1991; Noga 1993). Over a 3-day period the water temperature was increased from 10 to 16°C by progressively mixing degassed, heated well water with ambient temperature well water in a header tank, which supplied all six replicate fish tanks. Water flow rates to all the fish tanks were identical at 2.7 L·min^{-1}. Based on results in the control tanks, and to a lesser extent in treated tanks, we were successful in inducing a "saddleback" of necrotic skin, typical for severe columnaris disease, subjacent to the dorsal fin and from there along lateral line regions of the fish.

The six tanks were assigned into two location-matched groups. Within these two groups, one tank was assigned to be static treated twice weekly with hydrogen peroxide at 200 mg·L^{-1} for 1 h (hydrogen peroxide 6%, Drug Trading Company Ltd., Scarborough, Ont.), another tank with *N*-sodium-*N*-chloro-*p*-toluenesulfonamide at 10 mg·L^{-1} for 1 h (Chloramine-T, Syndel Laboratories, Vancouver, B.C.), and the third left as a control with water turned off for 1 h twice weekly. The trial ran for 16 days. The first treatment was on day 3 after fish were placed into the tanks, the second on day 7, the third on day 10, and the fourth on day 14.

To confirm the occurrence of columnaris, skin scrapings from affected areas were cultured onto Cytophaga media plates and incubated at two temperatures (15 and 20°C). Subsequently, dominant isolates were biochemically profiled and assessed with an IFA method as previously described.

To evaluate the treatments, a discontinuous fin scoring index (0, fin intact; 1, up to 25% missing; 2, 25–50% missing; 3, 50–75% missing; 4, 75–100% missing; 5, 100% missing and skin below dorsal fin with grossly detectable areas of necrosis) was used to grade the severity of the dorsal fin erosion and involvement of the epithelium of the dorsum subjacent to the dorsal fin. All fish from each tank were evaluated on day 1 after random allocation to tanks and at the conclusion of the trial on day 16. Tank effects were ruled out by evaluating fin scores (Mann–Whitney *U* test). Data from replicate tanks for each treatment were then combined and the presence of a treatment effect on fin scores assessed with the Kruskal–Wallis (KW) test. If the KW test indicated treatment effects, then specific treatments were compared with Dunn's multiple comparison test.

Effects of hydrogen peroxide exposure on growth

Rainbow trout juveniles (mean weight 3.5 g) were purchased from a local hatchery and subsequently housed in our aquatic animal facility. For the next 4 weeks they were acclimated to the water conditions, temperature (10°C), lighting cycle (12 h light : 12 h dark), commercial, fish meal based, salmonid diet, and feeding regime (twice daily to satiation) that would be used for the growth trial. Twenty fish were arbitrarily sampled, and tissues from each were examined histopathologically to preclude the presence of disease.

At the end of the acclimation period, 540 fish (mean weight 6.2 g) were arbitrarily selected and randomly allocated, in groups of 30, to each of 18 identical circular 100-L tanks. Each tank contained 70 L of water, a central draining standpipe, and an air-lift tube. The initial stocking density was 2.7 kg·m^{-3}, and by the end of the trial, was 6.2–6.7 kg·m^{-3}. The growth trial began 1 day after transfer to these tanks and ran for 7 weeks. Each tank was supplied with 2.7 L·min^{-1} of water.

Fish were fed to satiation by hand twice daily (weekdays), once on Saturday, and not on Sunday. We used an ad libitum without feed wastage method (Mäkinen 1994), with modified cut-off criteria as suggested by Vijayan and Leatherland (1988) to eliminate uneaten food (Speare et al. 1995*b*). Technical staff assigned to feed the fish were not aware of the treatment assignment of tanks.

In a previous growth study using this system of tanks, a significant location-dependent tank effect on growth was noted that could be controlled using a block design (Speare et. al. 1995*b*; Speare and MacNair 1996). Treatments were allocated evenly within blocks, by using nine location-matched pairs of tanks, one of each randomly selected to be a treatment tank.

Fish were treated twice weekly (Wednesday and Sunday) for a total of 14 treatments for each tank during the growth trial. A treatment consisted of stopping the water flow for 1 h, and adding 230 mL of 6% hydrogen peroxide diluted in about an equal amount of tank water to achieve a final tank concentration of 200 mg·L^{-1}. The hydrogen peroxide concentration of the commercial product, and tank concentrations at the start of a treatment were confirmed by an analytical testing service (Toxicology and Analytical Services, University of Prince Edward Island, Charlottetown, P.E.I.). A control tank was treated by turning off the water flow for 1 h (air-lift tube remained in operation) and sham treating it by manually removing and replacing a 500-mL bucket of water. At the end of 1 h, tank water flow was resumed.

The amount of feed eaten was recorded weekly and tallied for the growth trial. All fish from each tank were anaesthetized and weighed at the end of weeks 3 and 7. From this we calculated mean weight for each tank. For the entire 7-week period, as well as for the two intervals (week 0 – end of week 3; start of week 4 – end of week 7), we calculated specific growth rates (SGR) (Jørgensen and Jobling 1993) and feed conversion ratios (FCR) (Kentouri et al. 1994) for each tank of fish. Mean values for each growth index for each treatment represent the mean derived from values of the nine tanks assigned to that

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Table 1. Fin score evaluations (mean) and proportion of fish with saddle patch skin necrosis at the beginning and end of the trial with hydrogen peroxide and chloramine-T.

	Fin score		Proportion with SPN[a]	
Treatment or control	Initial	Final	Initial	Final
Hydrogen peroxide[b]	3.47	3.40*	2/60	6/60*
Chloramine-T[c]	3.31	3.78	2/60	14/60*
Control	3.48	4.10	1/60	25/60

Note: *, significantly different from controls ($P < 0.05$).
[a]Saddle-patch necrosis.
[b]200 mg/L for 1 h twice weekly.
[c]10 mg/L for 1 h twice weekly.

treatment. For each growth index, a matched-pairs *t*-test (Ostle and Mensing 1975) was used to test the null hypothesis that there was no treatment effect.

At the end of the growth trial, 2 fish were arbitrarily selected from each of the tanks (total of 18 treated and 18 control fish) and individually euthanized (100 mg·L^{-1} benzocaine). The second gill arch on the left side of the fish was quickly removed and placed in 10% phosphate-buffered formalin. The gill samples were routinely processed for histology, stained with haematoxylin and eosin, and observed with light microscopy for evidence of lesions typical for exposure to chemicals (Mallat 1985) or infectious disease (Speare and Ferguson 1989).

Data analysis

Differences were considered significant at $\alpha = 0.05$. Calculations were done with commercial statistical software packages (GraphPad/Instat/Prism, GraphPad Software, Inc., San Diego, Calif.). Assumptions of normality and homogeneity of variance were assessed for parametric calculations.

Results

Efficacy of hydrogen peroxide and chloramine-T for columnaris control

Of the 10 fish sampled at the beginning of the trial, medium to large quantities of Gram negative filamentous bacteria, which reacted positively for *F. columnaris* during IFA assessment, were noted in whole mounts and scrapings taken from the dorsal fin. The margins of the dorsal fins were eroded, and in a few cases, punctate areas of hemorrhage were present along the necrotic border. In tissue sections taken from the dorsal fin, filamentous bacteria were noted to be invading the dermis and lepidotrichia of the fin.

Flexibacter columnaris was cultured and identified by positive IFA evaluation from the dorsal fins of each of the eight fish sampled from the control group at the end of the trial. Histologically, all eight of them had extensive necrosis of the dorsal fin associated with the presence of invading filamentous bacteria. The epithelium immediately subjacent to the dorsal fin was affected in a similar fashion to the fin in three of the eight fish and markedly acanthotic but not colonized in the other five. In contrast, bacteria were not noted in tissue sections of any of the treated fish, and typically the epithelium at the truncated edges of the dorsal fin was thickened and acanthotic with numerous inflammatory cells present between epithelial cells.

At the beginning of the trial, there were no differences in the fin scores or the prevalence of saddle-patch skin necrosis between the two treatment groups (Table 1). The fin condition of hydrogen peroxide treated fish was significantly better ($P < 0.001$) than control fish at the end of the trial (Table 1); however, comparison of chloramine-T versus controls and hydrogen peroxide versus chloramine-T revealed no significant differences in fin condition. The proportion of fish with saddle-patch necrosis was significantly less in the hydrogen peroxide ($P = 0.0001$) and chloramine-T treated ($P = 0.05$) fish compared with controls. Although a lower proportion of the hydrogen peroxide compared with chloramine-T treated fish had saddle-patch skin necrosis, the difference was not significant ($P = 0.08$). One mortality occurred in a control tank on the final day of the trial. Fin score data for that fish were not recorded; however, based on its gross appearance and whole mounts from areas of skin erosion, the fish probably died from columnaris disease.

Table 2. Summary of growth indices (mean ± SE) for disease-free juvenile rainbow trout treated or not treated (control) with hydrogen peroxide for the first period of 3 weeks, the second period of 4 weeks, and the entire 7-week period.

Index	Interval	Treated	Control	*P*
Weight (g)	End of week 7	16.15±0.51	15.60±0.42	0.364
SGR[a]	Week 0 to week 3	1.43±0.09	1.73±0.11	0.043*
SGR	Week 4 to week 7	2.38±0.09	2.05±0.12	0.032*
FCR[b]	Week 0 to week 7	0.94±0.04	0.94±0.03	0.985
FCR	Week 0 to week 3	1.33±0.09	1.04±0.08	0.039*
FCR	Week 4 to week 7	0.89±0.07	0.93±0.05	0.610
Feed[c]	Week 0 to week 7	9.22±0.35	8.77±0.35	0.370
Feed	Week 0 to week 3	2.76±0.08	2.77±0.11	0.581
Feed	Week 4 to week 7	6.46±0.30	5.90±0.19	0.047*

Note: *, treated fish were significantly different from control fish.
[a]Specific growth rate.
[b]Feed conversion ratio.
[c]Amount of feed consumed per fish (g).

Growth trial

No more than one fish died per tank during the 7-week trial. Most tanks had no mortalities. All fish appeared healthy at the time of final assessment.

The mean weight of hydrogen peroxide treated fish was not significantly greater than untreated fish after 7 weeks (Table 2). However, there was significant growth suppression in the hydrogen peroxide treated fish during the first 3 weeks, in which the SGR of treated fish was significantly less than untreated fish (Table 2). The opposite trend occurred during the final 4 weeks, during which fish continued to receive treatment: the SGR of treated fish was significantly greater than that of untreated fish (Table 2).

The FCR of treated and untreated fish, when examined over the entire 7 weeks, did not differ (Table 2). However, during the first 3 weeks the FCR was significantly lower (i.e., more efficient) for untreated fish compared with treated fish. During the final 4 weeks, however, the FCR of the two groups did not differ (Table 2).

There was no significant difference in feed consumption by treated fish compared with untreated fish over the first 3-week period, or over the entire 7-week period (Table 2), although values for treated fish were slightly higher during both periods. However, during the final 4 weeks of the trial, treated fish

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consumed a significantly greater amount of food than untreated fish (Table 2).

The histological features of gills taken from treated fish at the end of the trial were identical to gills from control fish. Features that were specifically examined for, but that were not present in either group, included evidence of cellular damage, changes in stem-cell differentiation (mucous cell or chloride cell metaplasia), infiltration of inflammatory cells, edema (separation of the interstitial spaces of the lamellae), lamellar fusion, and epithelial hyperplasia.

Discussion

Only a few publications have addressed the efficacy of hydrogen peroxide as a treatment for fish diseases, but Roberts (1994 and 1995) and Rach et al. (1995) report a dose rate of between 50 and 250 $mg \cdot L^{-1}$ for 1 h as being safe and effective for several species of salmonids, either as single or repeatedly administered treatments. The water in their studies was similar to that in ours. Our choice of 200 $mg \cdot L^{-1}$ of hydrogen peroxide as the treatment dose was arbitrary, but was effective when repeated twice weekly. Hydrogen peroxide treatment at 200 $mg \cdot L^{-1}$ slightly outperformed chloramine-T at 10 $mg \cdot L^{-1}$ as a prophylactic agent for columnaris disease, although both provided significant protection.

In our study, the lack of fish mortality or changes to gill anatomy even after 14 intermittent treatments, supports the report by Rach et al. (1995) that hydrogen peroxide can be used safely for healthy juvenile rainbow trout. Additionally, there were no mortalities after peroxide treatment during our columnaris prophylaxis study. Although it is tempting to suggest that our dose range is also safe for sick fish, the fish used in our columnaris study are better defined as subclinically affected, since they were feeding and behaving normally at the time treatment was first initiated. The distinction between target-animal safety for sick and healthy fish is important. For example, formalin treatments with dose levels well tolerated by healthy fish can be fatal to fish with infections such as branchial costiasis (*Ichthyobodo necator)* (Tojo et al. 1994) and BGD (Ostland et al. 1995). Rach et al. (1995) noted that diseased walleye (*Stizostedion vitreum*) were more sensitive to the toxic effects of hydrogen peroxide treatment than healthy ones. Further studies need to address the tolerated dose for more severely diseased juvenile trout. Similarly, differing water conditions may affect the rate of dissociation and, hence, the oxidizing effect of hydrogen peroxide. For example, Bruno and Raynard (1994) found that temperature had a marked effect on the toxicity of high-dose peroxide to Atlantic salmon (*Salmo salar*).

Growth trials have the potential to determine subclinical, and potentially economically important, effects of chemical-based disease management strategies (Speare and MacNair 1996). However, we cannot infer that our results apply to typical production settings. For example, in our growth trial, tank conditions were optimized so that infectious diseases would not develop and affect growth indices. Given that disease is more likely to be a factor at commercially practical stocking densities and loading rates, the potential for hydrogen peroxide to prevent disease may offset the early treatment-related growth suppressive effect noted in our study. With respect to feeding practices, we mimicked commercial production in that fish were fed to satiation, a commercial ration was used, and acceptable growth rates were obtained (Cho 1992). If fish had not been fed to maximal levels, some of the effects of peroxide may have been not detected, particularly the compensatory growth trend, which was related to changes in appetite.

Compensatory or "catch-up" growth is a common feature in fish growth trials (Sumpter 1992; Mortensen and Damsgård 1993) and is a feature that should be specifically sought before drawing conclusions on the negative production effects of management strategies including treatments. In our trial, compensatory growth resulted in a similar final weight for treated fish compared with controls. Although there would be an economic effect relating to the poorer feed conversion of the peroxide-treated fish during the first 3 weeks of the trial, this would have to be put into its proper perspective. If peroxide treatments were begun during the early parts of a production cycle, when feed costs are low, the increased costs due to poorer feed conversion ratios would be small. This assumes that conversion ratios recover, and treated fish undergo compensatory growth as they did in our trial. Additional economic benefits would accrue if the peroxide treated fish have a lower incidence of disease.

Our study indicates that the mechanism through which feed conversion was inhibited was probably not persistent over time, and the fish adapted to the treatment protocol. A possibility is that treatment may have induced increased levels of protective cellular antioxidants, and their presence may have mitigated the free-radical oxidant properties of the treatment. In refutation of this hypothesis, at the conclusion of the growth trial we challenged these fish with higher concentrations of peroxide to see whether there was any acclimation or protection provided by prior exposure. Treatment history did not affect survival when fish were challenged for 1 h at 750, 1000, 1250, or 1500 $mg \cdot L^{-1}$ (unpublished data).

Differences in response of fish to the three chemicals most likely to be used in chemical prophylaxis regimes of freshwater cultured species need to be considered. Repeat prophylactic treatment with formalin has been shown to have no negative effect on the condition index of catfish (Bodensteiner et al. 1993), the growth of rainbow trout (Speare and MacNair 1996), or the growth and post-seawater transfer survival rates of chinook salmon (*Oncorhynchus tihawytscha*) or Atlantic salmon smolt (Smith et al. 1987; Powell et al. 1996). Additionally, repeat formalin treatment improved the fin condition of rainbow trout during a growth trial with fish at high stocking density (Speare and MacNair 1996). In contrast, in two separate studies, repeat treatment with chloramine-T had a persistent suppressive effect on the SGR of rainbow trout (Powell et al. 1994; Sanchez et al. 1996) specifically resulting from inhibited feed conversion indices, with no true compensatory growth phase even during an 11-week trial (Sanchez et al. 1996). Peroxide's effects on growth were initially similar to those of chloramine-T. However, the effects were reversed later, and fish performance was unaffected overall. Therefore, in consideration of growth dynamics, peroxide and formalin, in contrast to chloramine-T, appear to be better chemical prophylactic agents. These studies should be expanded through field trials to compare the relative abilities to restrict endemic diseases, since in the absence of dramatic growth performance effects, this may be the best criterion upon which to select a chemoprophylactic regime.

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Refinement and calibration of a bioenergetics-based foraging model for kokanee (*Oncorhynchus nerka*)

Jason D. Stockwell and Brett M. Johnson

Abstract: Results from a mechanistic, bioenergetics-based foraging model for kokanee (*Oncorhynchus nerka*) were compared with results from a corroborated standard model. Daily growth estimates from the mechanistic model were 239% higher than estimates from the standard model at temperatures ≥12°C, and 42% lower at temperatures ≤8°C. The mechanistic model was then modified by incorporating a different respiration function and a new size- and temperature-dependent functional response. Although sensitive to prey and predator size, prey handling time, and feeding duration, results from the modified model were comparable with those from the standard model. Using observed vertical profiles of temperature and prey densities, model growth estimates for kokanee from Blue Mesa Reservoir, Colorado, bounded observed growth under realistic ranges of model parameters. The model also made the following four predictions: seasonal and annual ontogenetic shifts in (*i*) foraging and (*ii*) migration strategies; (*iii*) very low prey handling times (0.33 s·*Daphnia*$^{-1}$) for larger fish, suggesting that kokanee may be capable of gulp or filter feeding; and (*iv*) higher daily maximum consumption rates for smaller kokanee than previously hypothesized. The revised model provides a mechanistic means to forecast anthropogenic and climatic thermal effects on fish behavior and growth.

Résumé : Nous avons comparé les résultats fournis par un modèle mécaniste et à base bioénergétique de l'alimentation du kokani (*Oncorhynchus nerka*) aux résultats fournis par un modèle standard corroboré. Les estimations de la croissance quotidienne obtenue grâce au modèle mécaniste étaient de 239 % supérieures aux estimations du modèle standard à des températures ≥ 12°C, et de 42 % inférieures à des températures ≤ 8°C. Nous avons alors modifié le modèle mécaniste en intégrant une fonction de respiration différente et une nouvelle réaction fonctionnelle dépendante de la taille et de la température. Bien qu'ils soient sensibles à la taille des proies et des prédateurs, au temps de manutention des proies et à la durée de l'alimentation, les résultats fournis par le modèle modifié étaient comparables à ceux du modèle standard. À l'aide des profils verticaux observés de la température et des densités de proies, les estimations modélisées de la croissance du kokani du réservoir Blue Mesa (Colorado) confinaient la croissance observée dans des plages réalistes des paramètres du modèle. Le modèle a aussi établi les quatre prédictions suivantes : modifications ontogéniques saisonnières et annuelles (*i*) de la stratégie d'alimentation et (*ii*) de la stratégie de migration; (*iii*) temps très court de manutention des proies (0,33 s · Daphnia^{-1}) pour les poissons de grande taille, ce qui permet de penser que le kokani peut être capable de se nourrir par engouffrement ou filtration; et, (*iv*) taux maximum quotidien de consommation plus élevé pour les petits kokanis qu'on ne le pensait jusque-là. Le modèle révisé constitue un outil mécaniste permettant de prévoir les effets sur le comportement et la croissance des poissons de l'action anthropique et du réchauffement climatique.
[Traduit par la Rédaction]

Introduction

Bioenergetics models (Hewett 1989; Hewett and Johnson 1992) have proven to be an invaluable tool in fisheries ecology. The elegance of these models (collectively called the "Wisconsin" model; Ney 1990) has been their use of fundamental thermodynamics to uncover important ecological insights. For example, the Wisconsin model has been used to explicitly predict physiological effects of body size (e.g., Post 1990), behavioral thermoregulation with changes in body size and food availability (e.g., Crowder and Magnuson 1983), and contaminant accumulation and elimination dynamics (e.g., Madenjian et al. 1993). Overall, these models have defined constraints on growth via temperature, body size, and feeding rate (e.g., Rice et al. 1983; Luecke et al. 1996). The Wisconsin model has also been useful for management purposes including determination of stocking densities (e.g., Stewart et al. 1981) and harvest rates (e.g., Carline et al. 1984; Johnson et al. 1992). For a complete review of the Wisconsin model, see volume 122(5) of *Transactions of the American Fisheries Society*.

Up to now, these models have performed well at predicting direct effects of environmental change on the consumption and growth of fishes. However, there are no explicit mechanisms for linking consumers with their prey (but see Madenjian et al. 1993). Feeding rate or growth is a required input to solve the energy budget. However, many environmental problems will affect predators indirectly through effects on their prey. For example, eutrophication, nutrient abatement, chemical

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pollution, thermal pollution, and climate change can affect production of lower trophic levels (e.g., Schindler et al. 1985; McQueen et al. 1986; Carpenter and Kitchell 1993; Porter et al. 1996, Zagarese et al. 1994). To predict the impact of these environmental effects, feeding rate needs to be an explicit, predicted function within the modeling framework.

Incorporation of an explicit feeding function is also important, because studies of predator–prey relationships are making a transition from aggregated population level analyses of predator demand and prey supply (e.g., Stewart et al. 1981; Stewart and Ibarra 1991; Eby et al. 1995; Rand et al. 1995) to more detailed, spatially explicit models of predator–prey interactions (e.g., Mason et al. 1995). These latter models scale up from individuals to populations while accounting for spatial heterogeneity in the biotic (e.g., prey distributions) and abiotic (e.g., temperature, light, and dissolved oxygen) environments.

In this paper, we refine and calibrate a spatially explicit, bioenergetics-based foraging model for kokanee salmon (*Oncorhynchus nerka*) (Bevelhimer 1990; Bevelhimer and Adams 1993) by incorporating an explicit and improved feeding function to link predator dynamics to prey density. Kokanee are well suited for this type of modeling approach because (*i*) they are extremely selective in their diet (e.g., Vinyard et al. 1982), (*ii*) their prey are usually vertically stratified (e.g., Lampert 1989), and (*iii*) they exhibit strong diel vertical migrations through a range of temperatures (e.g., Finnell and Reed 1969). Our goal was to develop a realistic and predictive model that could be used to study possible climatic and anthropogenic thermal effects on growth and production of kokanee. In addition to its utility for applied issues, the model provides a framework for examining basic foraging ecology of kokanee and other planktivorous fishes.

Methods

We first describe the model of Bevelhimer and Adams (1993) and outline the comparison we make with the Wisconsin model. Results of this comparison are presented to introduce and justify the refinements to Bevelhimer and Adams' model. We then outline further comparisons between the modified model and the Wisconsin model, and a preliminary sensitivity analysis of the modified model. Finally, we describe parameterization and calibration of the modified model using field data.

General model description

We adopted the bioenergetics model for kokanee developed by Bevelhimer and Adams (1993), hereafter referred to as the B&A model. The B&A model was originally developed to test the growth-maximization hypothesis for kokanee (Bevelhimer and Adams 1993). This hypothesis predicts that kokanee undergo diel migrations to maximize their net energy assimilation by maximizing food consumption and minimizing metabolic costs. Alternatively, Eggers (1978; sockeye salmon, the anadromous form of kokanee) and Johnston (1990; kokanee) suggest that diel migration by juveniles is a result of predator avoidance, whereas Clark and Levy (1988; sockeye) propose that migration results from a trade-off between predation risk and energetic efficiency. Results from Bevelhimer and Adams (1993) support the growth-maximization hypothesis when kokanee and their prey are thermally segregated; vertical migration can be energetically advantageous under these conditions.

The B&A model is based on the bioenergetics mass-balance equation (Kitchell et al. 1977):

$$(1) \qquad G = C - (R + F + U)$$

where G is the specific growth rate ($g \cdot g^{-1} \cdot day^{-1}$), C is the specific rate of consumption, R is the specific rate of respiration (including basal and active metabolism and specific dynamic action), F is the specific rate of egestion, and U is the specific rate of excretion. The B&A model differs from the Wisconsin model in the following four features: (*i*) it is constructed to estimate growth from consumption; (*ii*) it models consumption as a type-II functional response, modified by a temperature-dependent digestion function; (*iii*) it is rescaled for a 30-min time step; and (*iv*) it allows fish to migrate vertically. Data requirements for a 24-h simulation include vertical profiles of water temperature and prey density, initial fish mass, time the fish spends in each depth stratum, feeding status (feeding or not feeding) during each 30-min time step, and mean prey size. A factorial combination of depths and feeding durations is used to examine resultant growth from a suite of possible migration strategies for each given set of environmental conditions.

Estimates of consumption and growth rates and gross conversion efficiency from Bevelhimer and Adams (1993) were within the range expected for similarly sized kokanee feeding at high rations (Brett 1979). However, these model estimates have not been directly compared with field estimates or results from other models. For the more mechanistic B&A model to be applied to the same range of basic and applied questions that the Wisconsin model has proven useful, calibration and corroboration are critical next steps.

To run a simulation, a fish of a given size is placed in a particular depth stratum with a corresponding water temperature, prey density, and mean prey size. If the fish is feeding, the amount of food consumed is determined as a function of prey density at that depth. At the end of the first 30-min period, fish growth is determined by subtracting energetic losses from energy consumed (see table 1 of Bevelhimer and Adams (1993) for model equations), and the fish moves (or stays) to the next depth stratum as indicated by model input. Any food remaining in the stomach at the end of the time step is carried over to the next 30-min time step. Food consumed during this next time step, dependent on feeding status and prey density at that depth, is added to any undigested food in the stomach. If the amount of food in the stomach would exceed the stomach capacity (Table 1; Bevelhimer and Adams 1993), then the fish is considered satiated, and consumption during the 30-min period is set at the stomach capacity. As a result, food intake during a 30-min time step can be lower than the amount determined by the functional response. The process of calculating growth is repeated for this and all subsequent time steps. At the end of 24 h, growth is summed over all time steps to provide an estimate of daily growth. Each 24-h simulation was run until a steady state (ending stomach content mass equal to beginning stomach content mass) was reached (Bevelhimer and Adams 1993). Results from the steady-state runs were used in the analyses.

The Wisconsin model was also used to examine the bioenergetics of kokanee. This model has been corroborated with independently derived estimates of consumption and energy budgets for three populations of juvenile sockeye (Beauchamp et al. 1989). Therefore, we were interested in any departures of the B&A model results from the Wisconsin model results for a 24-h simulation, and we attempted to refine the B&A model to reconcile these differences.

We evaluated the B&A model by comparing the daily scope for growth (energy available to a fish after accounting for all energetic losses) and specific rates of consumption, respiration, egestion, and excretion of a 500-g kokanee to the Wisconsin model. The standard simulation of Bevelhimer and Adams (1993) was initially used for the B&A model. Inputs to the model for the standard simulation include (*i*) feeding duration of 12 h, (*ii*) feeding swimming speed of 20 $cm \cdot s^{-1}$, and (*iii*) 2.25-mm prey (*Daphnia*). The model was run for 24 h over a range of temperatures (4–25°C). Maximum scope for growth was estimated by setting prey density to a saturation level (10^6 *Daphnia*$\cdot m^{-3}$) where the fish could feed at maximum rate (73 *Daphnia*$\cdot min^{-1}$; Hyatt 1980). For the Wisconsin model (Hewett and Johnson 1992), parameters were taken from Beauchamp et al.

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Table 1. The modified B&A model as developed from Bevelhimer (1990) and Bevelhimer and Adams (1993).

Parameter	Value	Source
	Consumption (*C*)	
C (*Daphnia*·min^{-1})	$(EZ)/(1 + Ezh)$TL(60)	
E, volume searched (m^3)	$(\pi R_d^2)/3(3v^2 + u^2)/v$	1
TL, Thornton–Lessem function	$[(0.58e^{(0.21(T-3))})/(1 + 0.58(e^{(0.21(T-3))} - 1))]$ $[(0.5e^{(0.97(24-T))})/(1 + 0.5(e^{(0.97(24-T))} - 1))]$	2,3
R_d, reaction distance (m)	0.08	4
v, kokanee swimming speed (cm·s^{-1})	$9.9e^{0.04057T}M^{0.13}$	3
u, *Daphnia* swimming speed (cm·s^{-1})	0	
Z, prey density (*Daphnia*·m^{-3})		
h, handling time (s·*Daphnia*$^{-1}$)	0.33–1.2	
T, temperature (°C)		
M, kokanee mass (wet g)		
Stomach capacity (wet g)	M·[14.1 – 4.95log$_{10}$(M)]/100 for $M < 253.5$ g; 0.022M for $M \geq 253.5$ g	5
m, *Daphnia* mass (wet mg)	$0.052L_d^{3.012}$	6
L_d, *Daphnia* length (mm)		
	Digestion (*D*)	
D (cal·g^{-1})	$[((Cm - M_0/r)(1 - e^{-rt})) + (Cmt)]E_{dap}$	7
r, digestion coefficient	0.0140T – 0.0154	8
M_0, initial stomach content mass (wet g)		
t, model time step (min)	30	9
	Respiration (*R*)	
R (cal·g^{-1}·t^{-1})	$0.00143\ M^{-0.209}e^{(0.0867)}$ACTIVITY·(OXYCAL)·$t/t_{day}$	3
ACTIVITY	$e^{(0.0234VEL)}$	3
VEL	$9.9e^{(0.04057)}M^{0.13}$	3
OXYCAL, oxycaloric conversion factor (cal·g^{-1} O_2)	3241	
t_{day}, length of day (min)	1440	
	Egestion (*F*)	
F (cal·t^{-1})	$0.455T^{0.222}D$	10
	Excretion (*U*)	
U (cal·t^{-1})	$0.02337T^{0.580}(D - F)$	10
	Specific dynamic action (SDA)	
SDA (cal·t^{-1})	$0.14(D - F)$	11
	Energy density	
E_{dap} (cal·wet g^{-1} *Daphnia*)	586	12
E_{kok} (cal·wet g^{-1} kokanee)	1.851M + 1250 for $M \leq 196$ g; 0.1254M + 1588 for $M > 196$ g	10

Note: Sources are as follows: (1) Gerritsen and Strickler (1977), (2) Thornton and Lessem (1978), (3) Beauchamp et al. (1989), (4) Hyatt (1980), (5) Brett (1971), (6) Dumont et al. (1975), (7) Elliott and Persson (1978), (8) Brett and Higgs (1970), (9) Bevelhimer and Adams (1993), (10) Hewett and Johnson (1992), (11) Brett and Groves (1979), (12) Richman (1958).

(1989), with proportion (*P*) of maximum consumption (C_{max}) set to 1.0. Growth and specific rates of consumption and losses were estimated over the same range of temperatures as the B&A model. The same *Daphnia* and kokanee caloric contents were used in each model (Table 1).

Results from the comparison of the two models showed a large discrepancy in daily scope for growth estimates. Daily scope for growth from the B&A model was an average of 42% lower than the Wisconsin model at temperatures ≤8°C and an average of 239% higher at temperatures ≥12°C (Fig. 1A). The B&A model predicted positive growth across all temperatures (4–25°C), with an optimum growth of 4.03 g·day^{-1} at 16°C. The Wisconsin model predicted positive growth for temperatures ≤18°C, with an optimum growth of 3.17 g·day^{-1} at 8°C (Fig. 1A).

The discrepancy in growth estimates can be attributed to much lower active respiration rates in the B&A model (Figs. 1B and 1C). Across all temperatures examined, respiration estimates from the B&A model were, on average, 43% lower than estimates from the Wisconsin model. The B&A model also estimated higher consumption rates and showed a plateau in consumption at higher temperatures (Fig. 1B), whereas the Wisconsin model predicted lower consumption rates as well as a drop in consumption at temperatures >18°C (Fig. 1C).

All other components of the energy budget used in the B&A model (egestion and excretion; Bevelhimer and Adams 1993) were the same functions used in the Wisconsin model (Beauchamp et al. 1989). We therefore modified the respiration and consumption functions of the B&A model to produce growth estimates comparable with the corroborated Wisconsin model.

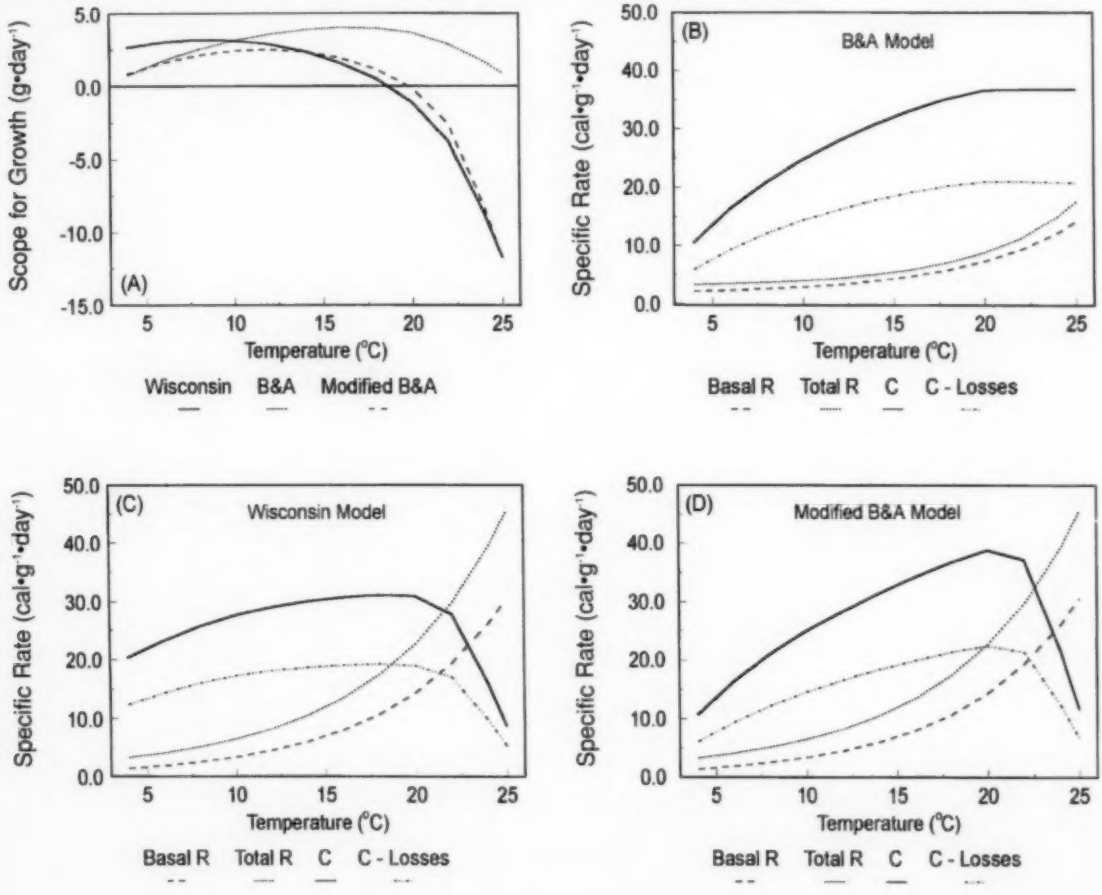
Modified B&A model

Respiration

The respiration function of Beauchamp et al. (1989; from Hewett and Johnson 1992)

$$(2) \qquad R = 0.00143\ M^{-0.209}e^{(0.0867)}\text{ACTIVITY}\cdot(\text{OXYCAL})\cdot\frac{t}{t_{day}}$$

Fig. 1. Comparison of estimates from the B&A, modified B&A, and Wisconsin bioenergetics models for a 500-g kokanee under standard simulation conditions (see text). (A) Scope for growth estimates as a function of temperature for all three models. (B) The energy budget from the B&A model as a function of temperature. Scope for growth is represented by area between total respiration (*R*) and consumption (*C*) minus losses curves. (C) and (D) Same as (B) but for the Wisconsin and the modified B&A models, respectively.



where R is respiration (cal·g^{-1}·t^{-1}), M is mass (g), T is temperature (°C), ACTIVITY = $e^{(0.0234 \cdot VEL)}$ and is the increment for active metabolism, VEL = $9.9 \cdot e^{(0.04057)} M^{0.13}$ and is the optimal swimming speed (cm·s^{-1}), OXYCAL is oxycaloric conversion factor (cal·g^{-1} O_2), t is the model time step (min), and t_{day} is the length of day (min), was substituted into the B&A model (Table 1). Respiration was divided by 48 to convert from the daily time step of Beauchamp et al. (1989) to the 30-min time step of Bevelhimer and Adams (1993).

Functional response

Experiments have demonstrated that young sockeye from the Pacific Northwest stop feeding as water temperature approaches their upper tolerance limit (≥23°C; Donaldson and Foster 1940; Brett 1952; Brett et al. 1969). We therefore concluded that the asymptote in consumption at the highest temperatures in the B&A model (Fig. 1B) was inappropriate for western North American stocks. To correct this, the amount of *Daphnia* biomass consumed in each time step was modified by Thornton and Lessem's (1978) temperature function (Beauchamp et al. 1989; Table 1). Application of the Thornton and Lessem function was scaled with reference to 10°C because the functional response used in the B&A model was derived at this temperature (Hyatt 1980; Bevelhimer and Adams 1993). Furthermore, the function was applied to the biomass of *Daphnia* consumed, and not the number consumed, because the function coefficients were derived using biomass measurements (Beauchamp et al. 1989).

Sensitivities of the Wisconsin model parameters are well known (Kitchell et al. 1977; Bartell et al. 1986). Therefore, we did not perform sensitivity analyses on the components of the modified B&A model shared with the Wisconsin model. However, we did perform a preliminary sensitivity analysis of the modified B&A model on the unshared components. The new model was run using two sizes of kokanee (100 and 500 g), three feeding durations (6, 12, and 18 h), and three prey sizes (1.75, 2.25, and 2.75 mm). Results were compared with the Wisconsin model across the same temperature range and maximum feeding rates used in the initial comparison.

Results from this comparison suggested one of two possibilities: either (*i*) there should be large differences in time spent foraging (~6 h) between 100- and 500-g fish or (*ii*) there are shortcomings in the consumption function. For the latter, consumption is the only plausible function to criticize as respiration, egestion, and excretion are identical in both models, and differences in SDA are both minimal and dependent on the amount of food consumed. Furthermore, the

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functional response used in the B&A model (Hyatt 1980) was derived from feeding experiments using a single size-class of kokanee (15 g) at 10°C and did not include the possibility of size-dependence in foraging rate. Because work on other fishes suggests predator size can influence their functional response (Werner 1977; Miller et al. 1992, 1993; Walton et al. 1994), we pursued modification of the functional response.

We scaled the functional response to predator size through volume searched using the following form of a Holling type-II functional response:

$$(3) \qquad C = \left(\frac{EZ}{1 + EZh}\right)60$$

where C is consumption (*Daphnia*·min^{-1}), E is volume searched (m^3), Z is *Daphnia* density (no.·m^{-3}), and h is handling time (s·*Daphnia*$^{-1}$; Table 1). Volume searched, E, was taken from Gerritsen and Strickler (1977):

$$(4) \qquad E = \frac{\pi R_d^2}{3}\left(\frac{3v^2 + u^2}{v}\right)$$

where R_d is the reaction distance of kokanee to their prey (m), v is kokanee swimming speed (m·s^{-1}), and u is *Daphnia* swimming speed (m·s^{-1}; Table 1). Kokanee swimming speed is size and temperature dependent and is determined from the VEL component of the respiration function (eq. 2; Table 1). We assumed that, during feeding, 100% of the *Daphnia* encountered by kokanee are ingested (Hyatt 1980; Vinyard et al. 1982).

To calibrate the functional response, eqs. 3 and 4 were used to predict consumption at the prey densities, fish size (15 g), and water temperature (10°C) used in the experiments of Hyatt (1980). Handling time was taken from the maximum consumption observed by Hyatt (~50 *Daphnia*·min^{-1} or 1.2 s·*Daphnia*$^{-1}$). Predictions from eqs. 3 and 4 under these conditions were then compared with the results of Hyatt (1980).

The new functional response scales for body size in two ways. First, increased body size increases swimming speed, thus directly influencing the volume searched E (eq. 4). The second possible influence is handling time differences among fish size-classes. No handling time data were available, so a range of values were used (Table 1). Reaction distance was assumed to be constant across the size-classes, because the literature suggests reaction distances in other visually feeding planktivorous fishes increase greatly during very early development, but then diminish beyond a certain size, usually within the first year of life (e.g., Breck and Gitter 1983; Rahmann et al. 1989; Wahl et al. 1993). Therefore, the mean reaction distance of 15-g kokanee from Hyatt (1980) was assumed to represent the reaction distance of larger fish (Table 1). We examined the sensitivity of the functional response to changes in fish body size (i.e., volume searched) and handling time.

Model parameterization and simulations

Growth estimates from the modified model were dependent on model inputs (predator and prey sizes, feeding duration, handling time). As a result, various combinations of model inputs could be used to generate growth estimates similar to the Wisconsin model. We therefore compared estimates of growth from the modified B&A model with observed growth of age-1, -2, and -3 kokanee from Blue Mesa Reservoir, Gunnison, Colo. (Cudlip et al. 1987). Inputs to the model included observed vertical profiles of temperature and *Daphnia* densities from 1994, mean prey size from kokanee stomachs sampled in 1995, and observed masses of fish from 1995. Limnological data from 1994 were used because these data were collected at a finer spatial and temporal resolution than in 1995. However, much more fish growth data were available from 1995 than 1994. Because comparisons of temperature profiles, prey densities and distributions, and kokanee growth collected in both years showed similar patterns (Johnson et al. 1995, 1996), we assumed results would not be compromised by using input from the two different years.

For simulations of each age-class, a range of feeding durations and handling times were used. The goal was not necessarily to match modeled growth with observed; inputs can be adjusted to generate matching results. Rather, the goal was to bound the estimates using a more mechanistic approach than the more commonly used Wisconsin model. Incorporating feeding mechanisms should then clarify which factors are most important to understanding kokanee growth and how these factors might direct future field and laboratory work.

Blue Mesa Reservoir (BMR) is a mesotrophic, 3700 ha, 32 km long impoundment in southwestern Colorado with a storage capacity of 1.16×10^9 m^3. The fish community of BMR is relatively simple, consisting of primarily kokanee, rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), lake trout (*Salvelinus namaycush*), and longnose sucker (*Catostomus catostomus*). Kokanee is the dominant fish species. However, the population has been steadily declining over the past 3 years with a concomitant increase in the lake trout population (S. Hebein, Colorado Division of Wildlife, Gunnison, unpublished data).

Thermal profiles of BMR were obtained using a Yellow Springs Instruments model 58 meter with a 60-m probe cable. Measurements were taken at 1-m intervals from 0 to 20 m and at 5-m intervals from 20 to 55 m, from May through September 1994.

Zooplankton were collected by oblique tows using a Wildco model 37-315 Clarke–Bumpus plankton sampler with a 130 mm diameter opening and a 153-μm net. The flowmeter on the Clarke–Bumpus sampler was calibrated using a Schwaffer water velocity meter. Two replicate samples were taken from each of 0–5, 5–10, 10–15, and 15–30 m strata on 21 May 1994. During June–September 1994, three replicate samples were collected every 2–3 weeks from each of 0–5, 5–10, and 10–15 m strata, and two replicates from the 15–30 m stratum. All samples were taken between 09:00 and 12:00 and preserved in 8% sugared, buffered formalin.

A 1-mL aliquot was taken from each sample using a Hensen–Stempel pipette. The aliquot was placed in a Sedgwick–Rafter cell, where all *Daphnia* were identified to species and enumerated (Lind 1979; Soranno and Knight 1993) under a compound microscope. The first 24 individuals encountered in each sample were measured with an ocular micrometer to the nearest 0.01 mm. Two aliquots from each replicate sample were processed.

Densities of *Daphnia* ≥1.0 mm in each stratum (m^3) were used as prey input to the model because (*i*) kokanee feed exclusively on *Daphnia* in BMR (97% *D. pulex* and 3% *D. galeata*; Johnson et al. 1995, 1996) and (*ii*) the original functional response used by Bevelhimer and Adams (1993) was a function of the density of *Daphnia* ≥1.0 mm (Hyatt 1980).

Mean length of each age-class of kokanee in 1995 was estimated from otolith samples obtained on 8 June (age 1, $n = 11$; age 2, $n = 20$; age 3, $n = 8$) and 26 July (age 1, $n = 22$; age 2, $n = 33$; age 3, $n = 16$) from fish sampled in vertical gill nets and angler's creels (Martinez 1996). Mean length at the end of the growing season was estimated from back calculations (age 1, $n = 123$; age 2, $n = 54$; Johnson et al. 1996) and from otoliths of fish in the spawning run (age 3, $n = 163$; Martinez 1996). Wet masses were computed using a length–mass regression ($r^2 = 0.95$, $n = 228$) developed from all fish sampled in vertical gill nets during June through September (Johnson et al. 1996). We computed the instantaneous daily growth rate (G) and interpolated masses (M) between fish sampling dates using the formula

$$(5) \qquad M_1 = M_0 e^{Gt}$$

where M_1 is the final mass (g), M_0 is the initial mass, and t is the number of days between sampling dates (for G) or number of days to the interpolated mass (for M). We estimated daily growth rates (DGR; g·day^{-1}) on limnological sampling dates from the equation

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(6) $DGR = M_0 e^{Gt} - M_0$

where M_0 is the mass at the start of the day, and $t = 1$ day.

Vertical profiles of temperature and *Daphnia* (≥1.0 mm) densities from a reference station in Sapinero Basin, the largest and most downstream basin of BMR, were used as inputs to the model (Table 1). Three dates were chosen from the 1994 sampling period to represent seasonal fluctuations in temperature and prey availability. Because mature BMR kokanee typically begin their spawning migration in September, we did not use September sampling dates for the simulations.

Temperature data on each limnological sampling date, t_{lim}, were averaged into seven depth strata: 0–5, 5–10, 10–15, 15–20, 20–25, 25–30, and 30–50 m. *Daphnia* densities from the zooplankton sampling were assigned to these strata accordingly. The observed densities from the 15–30 m strata were consistently low and were assigned to each of the 15–20, 20–25, 25–30, and 30–50 m strata. The 30–50 m stratum was used to allow kokanee access to deeper, colder water despite no routine zooplankton sampling at these depths. Few *Daphnia* were found in zooplankton samples collected from 40 and 50 m in July 1994 and 1996 (B.M. Johnson, unpublished data), indicating extrapolation of *Daphnia* densities from the 15–30 m stratum to these depths is a reasonable assumption. Additionally, data from experimental vertical gill nets set in BMR in 1994 and 1995 show that kokanee do reside at these depths at times (Johnson et al. 1995, 1996).

Daphnia do not vertically migrate in BMR (Johnson et al. 1995). Therefore, the vertical distribution of *Daphnia* used as model input for each 24-h simulation was held stationary.

Fish were allowed to locate at two depths (feeding and nonfeeding periods) in each 24-h simulation. Depths were the midpoints of the strata identified above (2.5, 7.5, 12.5, 17.5, 22.5, 27.5, and 40 m). All possible migration strategies were evaluated (7 depth strata = 28 different migration strategies). The migration strategy that maximized growth on each limnological sampling date (t_{lim}) was noted, and that growth was used in the comparison with the observed growth estimates on that date.

The mean size of *Daphnia* in the diets of BMR kokanee in 1995 (1.68 mm; Johnson et al. 1996) was used to convert consumption from numbers to biomass (Table 1). Mean size consumed did not differ across the season or age-classes (B.M. Johnson, unpublished data). Initial masses of each kokanee age-class were determined for day $t_{lim} - 1$ using eq. 5. Very little information on kokanee feeding duration and prey handling times was available in the literature, but initial growth estimates from the model appeared to be sensitive to these parameters. We performed simulations at seven different feeding durations (4, 6, 8, 10, 12, 14, and 16 h) and five different handling times (0.33, 0.5, 0.67, 1.0, and 1.2 s·*Daphnia*$^{-1}$) to evaluate the sensitivity of the modified model to these parameters. We used observed growth ±20% as our criteria for determining which combinations of feeding durations and handling times were realistic.

Results

Modified B&A model

For a 500-g kokanee under standard simulation conditions, application of the respiration function of Beauchamp et al. (1989) and the Thornton and Lessem (1978) temperature function greatly improved the performance of the B&A model when compared with the Wisconsin model. The combination of higher respiration rates (identical to the Wisconsin model) and the decline in consumption at higher temperatures (Fig. 1D) brought estimates of scope for growth in line with estimates from the Wisconsin model for temperatures >10°C (Fig. 1A). Growth estimates at low temperatures were still less than the Wisconsin model, a result of lower consumption in the modified B&A model at these temperatures (Fig. 1D).

Scope for growth varied considerably with fish size and feeding duration when prey size (2.25-mm *Daphnia*) was held constant (Figs. 2A and 2B). For a 500-g kokanee, a 12-h feeding duration best fit growth estimates from the Wisconsin model (mean squared error (MSE) = 0.82; Fig. 2A). A 6-h feeding period resulted in lower estimates across the entire temperature range (MSE = 3.97), while an 18-h feeding period resulted in higher estimates (MSE = 5.85; Fig. 2A). Temperature for optimum growth also shifted with changes in feeding duration. The more feeding duration was restricted, the lower the optimum temperature (Fig. 2A). This is expected from the B&A model; as food becomes limited, simulated fish minimize energy costs by using colder water (Bevelhimer and Adams 1993). This has also been demonstrated empirically (Crowder and Magnuson 1983). For the 100-g kokanee, a 6-h feeding duration best matched growth estimates from the Wisconsin model (MSE = 0.10; Fig. 2B). Feeding periods of 12 and 18 h both overestimated scope for growth (MSE = 1.27 and 4.27, respectively). Again, a decrease in optimum temperature for growth occurred with decreasing feeding durations (Fig. 2B).

When prey size and feeding duration were varied simultaneously, and fish were held at their optimum temperature for growth, results differed for the two fish sizes. Increasing prey size from 1.75 to 2.75 mm (an increase in *Daphnia* mass of nearly 400%) for the 100-g fish resulted in relatively small increases in growth (average across feeding durations = 12%; Fig. 2D). Much larger increases occurred when feeding time was increased from 6 to 18 h (average across prey sizes = 218% increase). For the 500-g fish, an increase in prey size from 1.75 to 2.75 mm translated to an average increase in growth of 458% (Fig. 2C). Increasing feeding duration for the larger fish also increased growth rates by over one order of magnitude (average of 1033%; Fig. 2C).

Estimates of consumption (*Daphnia*·min^{-1}) from the functional response using eqs. 3 and 4 for a 15-g kokanee, feeding at 10°C with a handling time of 1.2 s·*Daphnia*$^{-1}$, fell within the 95% confidence intervals of the experimental data of Hyatt (1980; Fig. 3A). Consequently, we assumed that the new functional response (eqs. 3 and 4) was adequate to describe foraging of a 15-g kokanee under these conditions and that it could then be scaled for body size effects via size-dependent search volume. A complete list of equations used in the modified B&A model is listed in Table 1.

Consumption rate estimates from the new functional response were much more sensitive to handling time than fish body size (Fig. 3B). The asymptote of consumption rate decreased by 70% (from 166 to 49 *Daphnia*·min^{-1}) when handling time was increased 260% (from 0.33 to 1.2 s·*Daphnia*$^{-1}$), whereas there was very little difference in consumption rate with a 90% reduction in body size (from 573 to 59 g; Fig. 3B).

Model calibration

Surface temperatures in Sapinero Basin in 1994 ranged from a low of 13°C on 21 May to a high of 20°C on 18 August (Fig. 4). Hypolimnetic temperatures generally stayed near 5°C throughout the season. Despite these vertical gradients, a strong thermocline was not apparent on any of the sampling dates (Fig. 4).

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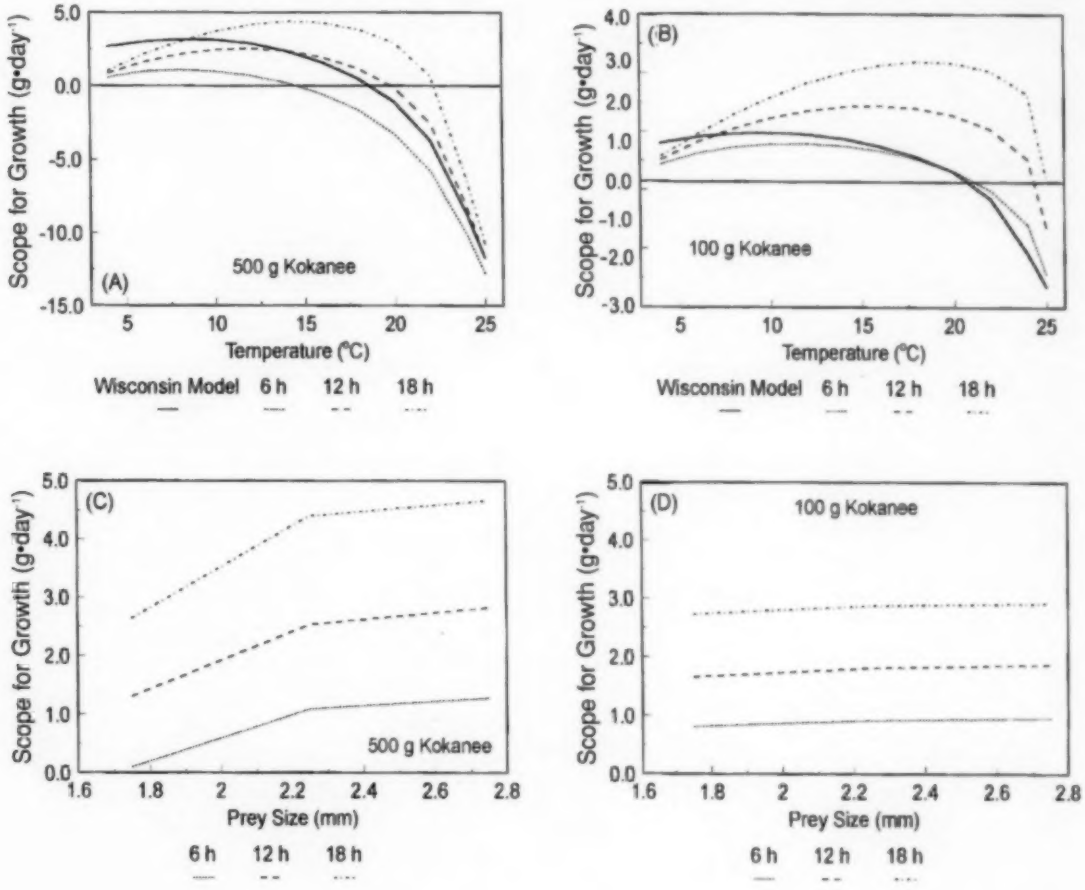
Fig. 2. (A) Scope for growth estimates for a 500-g kokanee as a function of temperature and feeding duration for the modified B&A model. Scope for growth estimates from the Wisconsin model are also shown. (B) Same as (A) but for a 100-g kokanee. (C) Scope for growth estimates for a 500-g kokanee as a function of feeding duration and prey size for the modified B&A model. (D) Same as (C) but for a 100-g kokanee.



The abundance of *Daphnia* ≥1.0 mm in the upper 10 m of the water column remained high throughout the season, accounting for 91% of all *Daphnia* in this size-class in the water column (Fig. 4). Maximum densities never fell below 9100·m^{-3} (21 May), while the highest recorded density exceeded 17 300·m^{-3} (21 July; Fig. 4). These maximum densities were well above densities required to saturate the functional response for each handling time (Fig. 3).

Growth rates of kokanee collected from BMR in 1995 were relatively high. Mean length of BMR kokanee at age 1 (170 mm; Johnson et al. 1996) was greater than back-calculated length at age 1 reported in other lakes throughout the western United States (range 78–156 mm, $n = 8$; Bjornn 1961; Bowler 1976, 1979; Cordone et al. 1971; Clark and Traynor 1972; Hanzel 1974*a*, 1974*b*). Age-2 and -3 BMR kokanee each had the second largest length at age (264 and 335 mm, respectively; Johnson et al. 1996) when compared with similarly aged fish throughout the west (age 2, range 154–319 mm, $n = 8$; age 3, range 209–362 mm, $n = 7$; Bjornn 1961; Bowler 1976, 1979; Cordone et al. 1972; Clark and Traynor 1972; Hanzel 1974*a*, 1974*b*). Daily growth estimates of BMR kokanee ranged from 0.41 to 1.39 g·day^{-1} for age-1 fish, from 0.52 to 2.66 g·day^{-1} for age-2 fish, and from 1.85 to 2.40 g·day^{-1} for age-3 fish (Fig. 5). All three age-classes showed a drop in daily growth over the second half of the field season (Fig. 5).

Simulations using the modified model (Table 1) and observed data from BMR (Figs. 4 and 5) corroborated results from earlier evaluations (Figs. 2 and 3). Daily growth estimates from the model indicated that different foraging strategies should be employed by different age-classes but that these optimal strategies change seasonally with changes in prey and temperature distributions and fish body size.

Early in the growing season (10 June), daily growth estimates of age-1 kokanee were more sensitive to changes in feeding duration than to changes in handling times (Fig. 6A). Using the mean growth rate across all handling times for each of the 4-h (0.66 g·day^{-1}) and 16-h (1.96 g·day^{-1}) feeding

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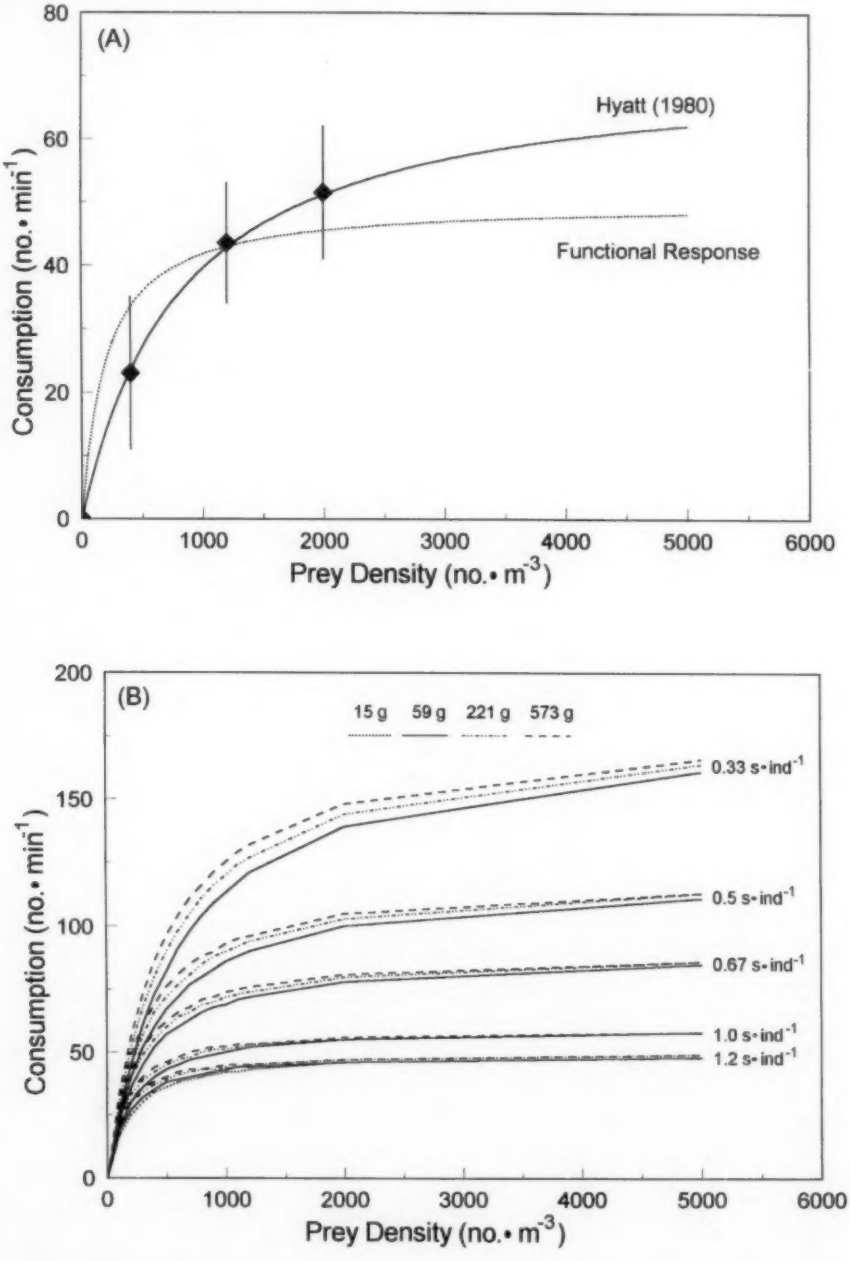
Fig. 3. (A) Comparison of original (Hyatt 1980) and modified functional responses. Hyatt's (1980) functional response was determined using laboratory experiments with 15-g kokanee at 10°C. The solid line describes the functional response by $C = ZI(0.0138Z + 11.5)$, where C is consumption (*Daphnia*·min^{-1}) and Z is *Daphnia* density (no.·m^{-3}). The dotted line indicates the modified functional response (see Table 1), using a 15-g kokanee at 10°C, with a handling time of 1.2 s·*Daphnia*$^{-1}$. (B) Sensitivity of the modified functional response when fish body size and handling times are varied.



durations (Fig. 6A), the range in daily growth estimates was 163% of the observed growth (0.80 g·day^{-1}). Using the mean growth rate across all feeding durations for each of the 1.2 s·*Daphnia*$^{-1}$ (1.22 g·day^{-1}) and 0.33 s·*Daphnia*$^{-1}$ (1.39 g·day^{-1}) handling times, the range in daily growth estimates was only 21% of the observed growth. Similar comparisons for age-2 and -3 kokanee showed that modeled growth of age-2 fish was also more sensitive to feeding duration than to handling time (90 versus 36%, respectively; Fig. 6B), whereas modeled growth of age-3 fish was more sensitive to handling time than

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Fig. 4. Vertical profiles of temperature and densities of *Daphnia* ≥1.0 mm in Sapinero Basin, Blue Mesa Reservoir, 1994. Asterisks indicate data used as inputs to the modified B&A model for comparison of model growth estimates with field observations.

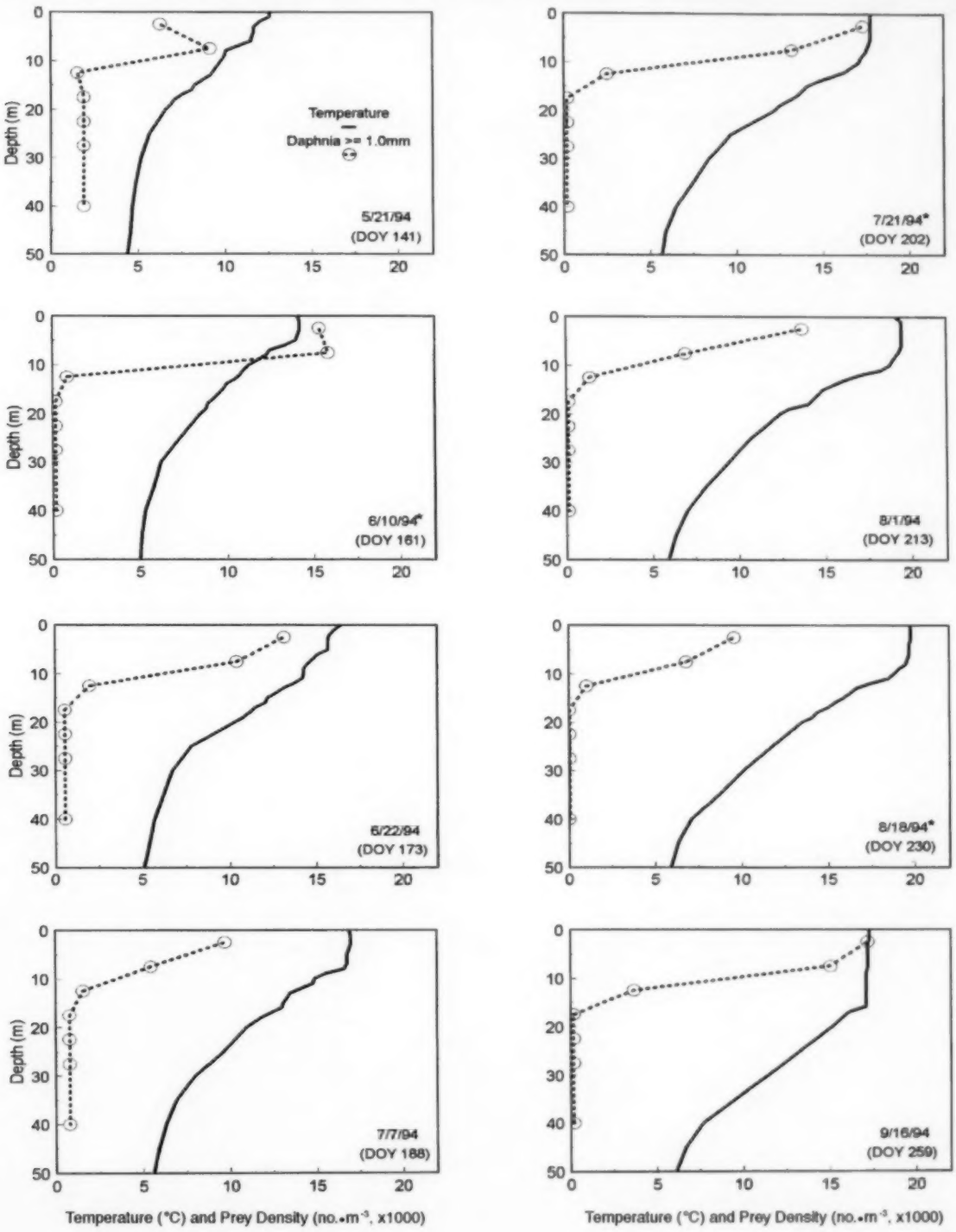

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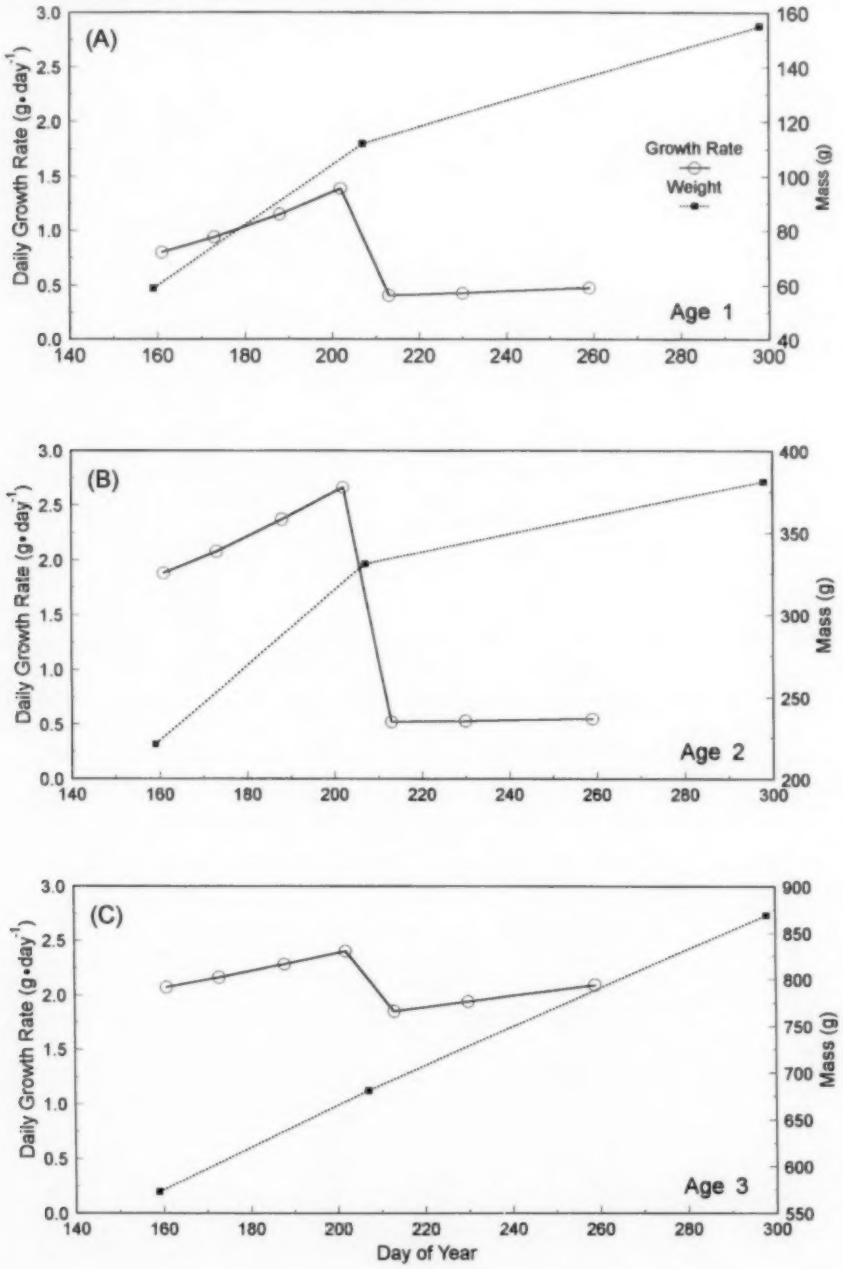
Fig. 5. Observed masses and daily growth estimates of (A) age-1, (B) age-2, and (C) age-3 kokanee from Blue Mesa Reservoir, 1995. Exponential growth was used to generate daily growth estimates (see text). Daily observed growth is plotted on limnological sampling dates (Fig. 4) to compare with estimates generated by the modified B&A model.



to feeding duration (151 versus 127%, respectively; Fig. 6C). Based on observed daily growth estimates (±20%), model simulations for 10 June suggest that age-1 kokanee should feed between 4 and 6 h·day^{-1}, age-2 between 12 and 16 h·day^{-1}, and age-3 between 8 and 16 h·day^{-1}, depending on the handling time (Fig. 6).

The model also indicated a progression in kokanee migration strategies for 10 June. Age-1 fish were predicted to feed at the surface and migrate the least, age-2 fish were predicted to feed at or near the surface and migrate slightly deeper than age-1 fish, and age-3 fish were predicted to feed below the surface and migrate the deepest (Fig. 6). Within each

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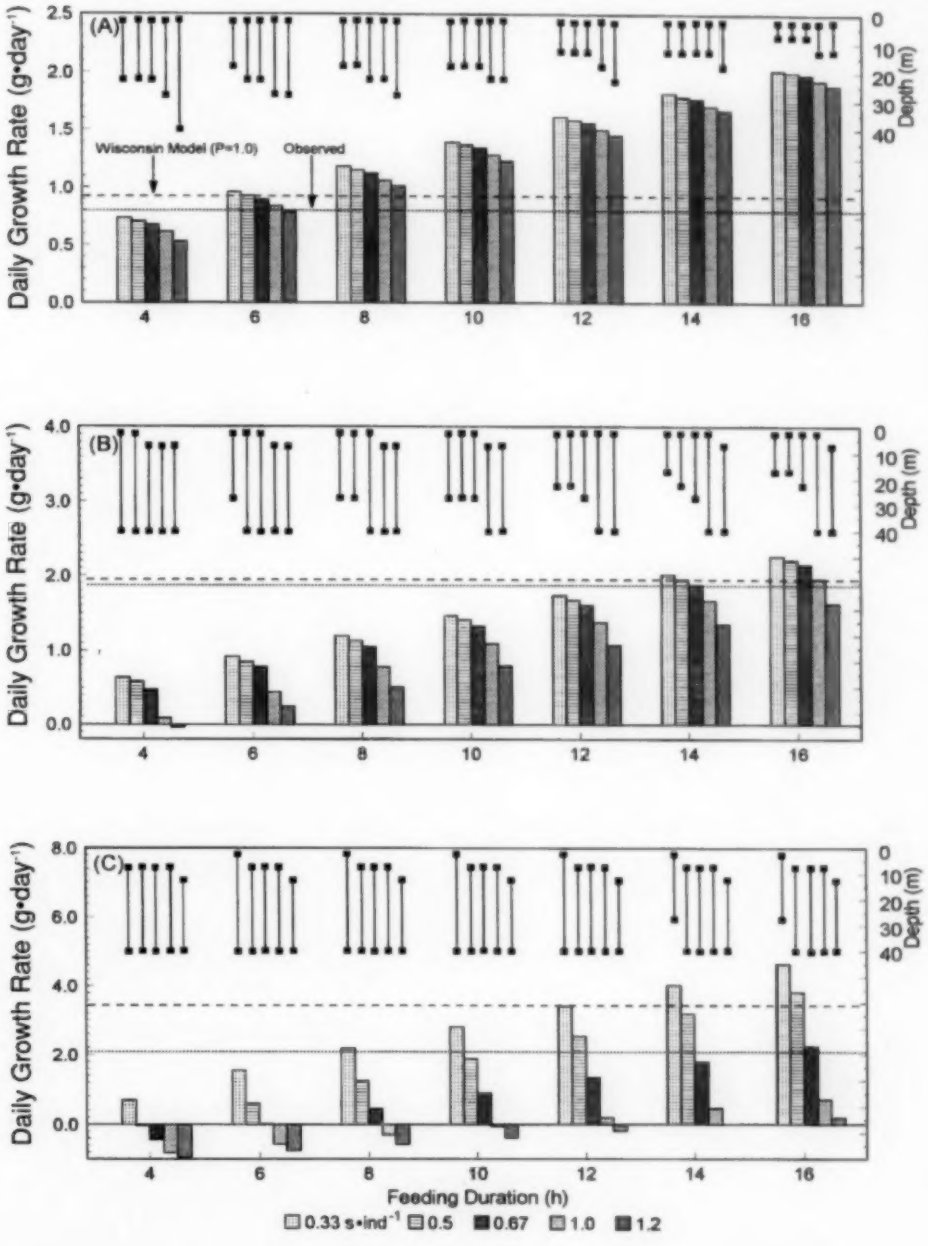
Fig. 6. Daily growth estimates from the modified B&A model for (A) age-1, (B) age-2, and (C) age-3 kokanee, using limnological data from 10 June 1994. Starting masses of kokanee for model simulations, as determined from field observations, were 59.79 g for age 1, 222.87 g for age 2, and 575.07 g for age 3. The horizontal dotted lines represent daily growth from field observations for the same date, while the horizontal broken lines indicate maximum scope for growth at optimum temperature as predicted by the Wisconsin model. Solid squares connected by vertical lines indicate depths of migration strategies from the modified B&A model that maximized growth for the given feeding duration and handling time.



age-class, foraging occurs higher in the water column and migration distances decrease with increases in feeding durations and (or) decreases in handling times (Fig. 6).

Daily growth estimates from the modified B&A model for age-1 kokanee during the middle of the growing season (21 July) were again more sensitive to feeding duration than

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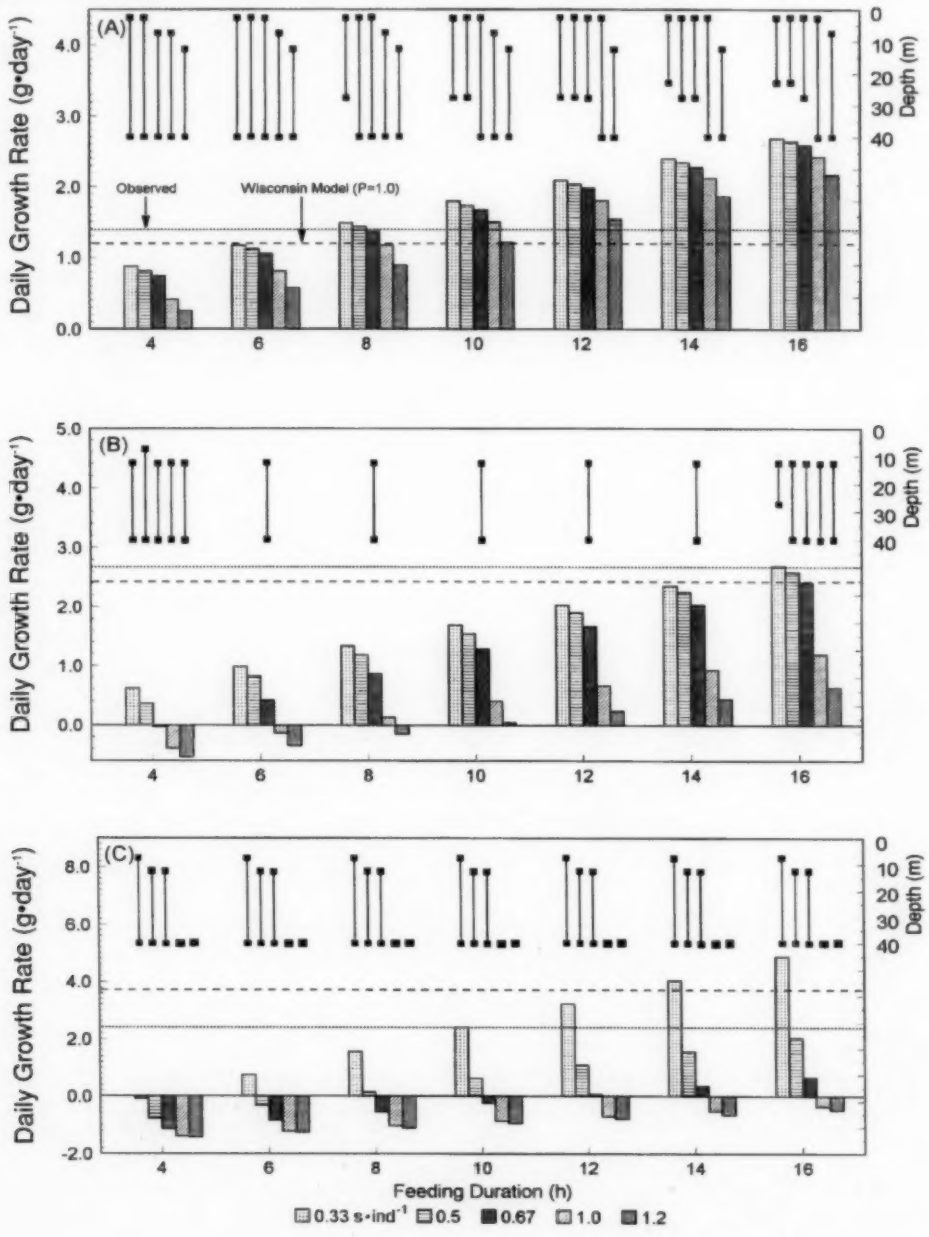
Fig. 7. Same as Fig. 6 but for limnological sampling date 21 July 1994. Starting kokanee masses for simulations were 103.38 g for age 1, 314.70 g for age 2, and 666.46 g for age 3. Single solid square for a given feeding duration and handling time indicates the migration strategy that maximized growth was to remain at the indicated constant depth for the entire 24-h simulation.



to handling time. Increasing feeding duration from 4 to 16 h changed daily growth by 135% compared with observed growth, whereas decreasing handling time from 1.2 to 0.33 s·*Daphnia*$^{-1}$ changed daily growth by 47% (Fig. 7A). Results (not shown) from simulations using the starting mass for age-1 fish on 10 June (60 g) for the 21 July input data (Fig. 4) were qualitatively similar to growth estimates from 10 June. This indicates that the increasing (decreasing) sensitivity of growth rates to handling time (feeding duration) for age-1 fish is a result of increased body size rather than differences in the prey and temperature distributions on the two dates. Model growth rate estimates for age-2 fish on 21 July were nearly

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equally sensitive to changes in feeding duration and handling time (71 versus 61%, respectively; Fig. 7B). Age-3 fish were more sensitive to handling time than to feeding duration (140 versus 96%, respectively; Fig. 7C), similar to simulation results for 10 June (Fig. 6C).

Comparisons of modeled growth estimates with observed estimates (±20%) on 21 July show that an increase in the amount of time spent feeding (compared with 10 June) was necessary for all age-classes to bound observed growth (Fig. 7). Age-1 fish needed to feed 6–12 h·day^{-1} to grow the observed 1.39 ± 0.28 g·day^{-1}; age-2 fish, 14–16 h·day^{-1} to grow 2.66 ± 0.53 g·day^{-1}; and age-3 fish, 10–16 h·day^{-1} to grow 2.4 ± 0.48 g·d^{-1} (Fig. 7). If the handling time of age-3 fish was restricted to ≥0.5 s·*Daphnia*$^{-1}$, their modeled growth would only approach the observed estimate if they fed for 16 h·day^{-1}, demonstrating the importance of an accurate handling time estimate for larger fish (Fig. 7C). Furthermore, simulations using the Wisconsin model for age-1 and -2 kokanee on this date show that these fish could not reach their observed growth when allowed to feed at maximum consumption (P = 1.0) at their optimum temperature (Figs. 7A and 7B).

In general, results from the modified B&A model also show that age-1 fish should forage at and migrate to deeper depths on 21 July than on 10 June. Age-1 fish will also feed closer to the surface and migrate less with increased feeding durations and (or) decreased handling times. The migration strategy of age-2 fish was to feed and migrate deeper than on 10 June. However, their migration strategy generally remained unchanged on 21 July regardless of feeding duration or handling time (Fig. 7B). The migration strategy of age-3 fish on 21 July remained unchanged with feeding duration but changed drastically with different handling times. For example, these fish migrated from 7.5 to 40 m with a handling time of 0.33 s·*Daphnia*$^{-1}$. At the highest handling times, they did not migrate, remaining at 40 m depth (Fig. 7C).

Results from model simulations for 18 August show that growth of age-1 fish was still more sensitive to feeding duration than to handling time (442% versus 212%, respectively; Fig. 8A) but that the difference was smaller than previous dates. Model growth of age-2 and -3 fish was more sensitive to changes in handling time than to changes in feeding duration (398% versus 336%, respectively, for age 2; 142% versus 24%, respectively, for age 3; Figs. 8B and 8C). Depending on handling time, age-1 kokanee needed to feed between 6 and 14 h·day^{-1} to bound the observed growth rate; age-2 fish, between 6 and 16 h·day^{-1}; and age-3 fish, between 12 and 16 h·day^{-1} (Fig. 8). The required feeding duration of age-2 fish dropped dramatically, which is a result of the substantial drop in the observed growth rates (from >2.5 to <1.0 g·day^{-1}; Fig. 5B).

Optimal migration strategies for 18 August, when surface water temperature was highest, show that age-1 fish will feed at and migrate to deeper depths than on 21 July. However, migration strategies within this age-class did not change very much with feeding duration or handling time (Fig. 8A). Migration strategies for age-2 and -3 fish are similar to their respective migration patterns on 21 July (Figs. 8B and 8C).

Discussion

In this study, we have modified the original B&A model to provide comparable results with the corroborated Wisconsin model and to calibrate the new model using field observations. The process of evaluating the sensitivity of the model and identifying combinations of inputs that bounded observed growth uncovered some interesting predictions about the foraging ecology of kokanee. First, the model predicted an ontogenetic shift in foraging strategy when kokanee reached approximately 300 g. Foraging strategies of kokanee <300 g should be to increase their feeding time, while strategies for fish >300 g should be to reduce their handling times. This shift in feeding strategy is a result of changing stomach capacities of the fish. If we assume that consumption of larger prey is equivalent to more efficient consumption of smaller prey, then the preliminary sensitivity analysis (Figs. 2C and 2D) demonstrates the relationship between stomach capacity and foraging strategy. While feeding on the smallest prey sizes (1.75 mm), the stomach capacity of smaller fish was reached quickly while consuming at maximum rates. The only way to increase consumption (i.e., growth) is to spend more time feeding. Conversely, larger fish were not limited by stomach capacity while feeding on 1.75-mm *Daphnia*. Therefore, increasing prey size to 2.25 mm (or increasing the rate at which 1.75-mm *Daphnia* are consumed) resulted in a much larger increase in growth.

Second, the model results suggested that age-3 kokanee required very short handling times (0.33 s·*Daphnia*$^{-1}$) to allow sufficient consumption rates to match their observed daily growth later in the season. Laboratory observations made by Hyatt (1980) demonstrated that age-0 kokanee are particulate feeders. However, it is difficult to accept that kokanee could feed on 3 *Daphnia*·s^{-1} with this feeding mode. Such a short handling time suggests the ability to forage by gulping and (or) filtering, and simple calculations show that this hypothesis is tenable. Mouth gape area measurements made on adult kokanee (mean mass 511.2 g) collected from BMR averaged 0.0004 m^2 (J.D. Stockwell, unpublished data). At 10°C, these fish have an optimum swimming speed of 0.33 m·s^{-1} (Beauchamp et al. 1989, Table 1). Using mean prey density of 17 500 *Daphnia*·m^{-3} (maximum observed densities in BMR; Fig. 4), and the equation

$$\text{(7)}\quad \text{Handling time} = \frac{1}{\text{mouth area} \times \text{swiming speed} \times \text{prey density}}$$

potential in situ handling time is estimated to be 0.43 s·*Daphnia*$^{-1}$. Given the integrated nature of zooplankton samples collected by the Clarke–Bumpus, it is likely that kokanee can experience much higher densities than 17 500 *Daphnia*·m^{-3} in BMR. Consequently, handling time could easily reach 0.33 s·*Daphnia*$^{-1}$.

While Hyatt (1980) did not observe filter feeding in kokanee during feeding experiments using *Daphnia* spp., maximum prey densities used in his experiments were 2000 · m^{-3}, compared with epilimnetic densities of 9000 to 17 500 · m^{-3} in BMR. We are not aware of any other studies that have specifically examined the foraging behavior of kokanee at such high zooplankton densities. Some studies have indicated that prey intake by other planktivorous fishes can be maximized by switching from particulate to filter feeding when prey concentrations exceed some critical threshold (e.g., Gibson and Ezzi 1992).

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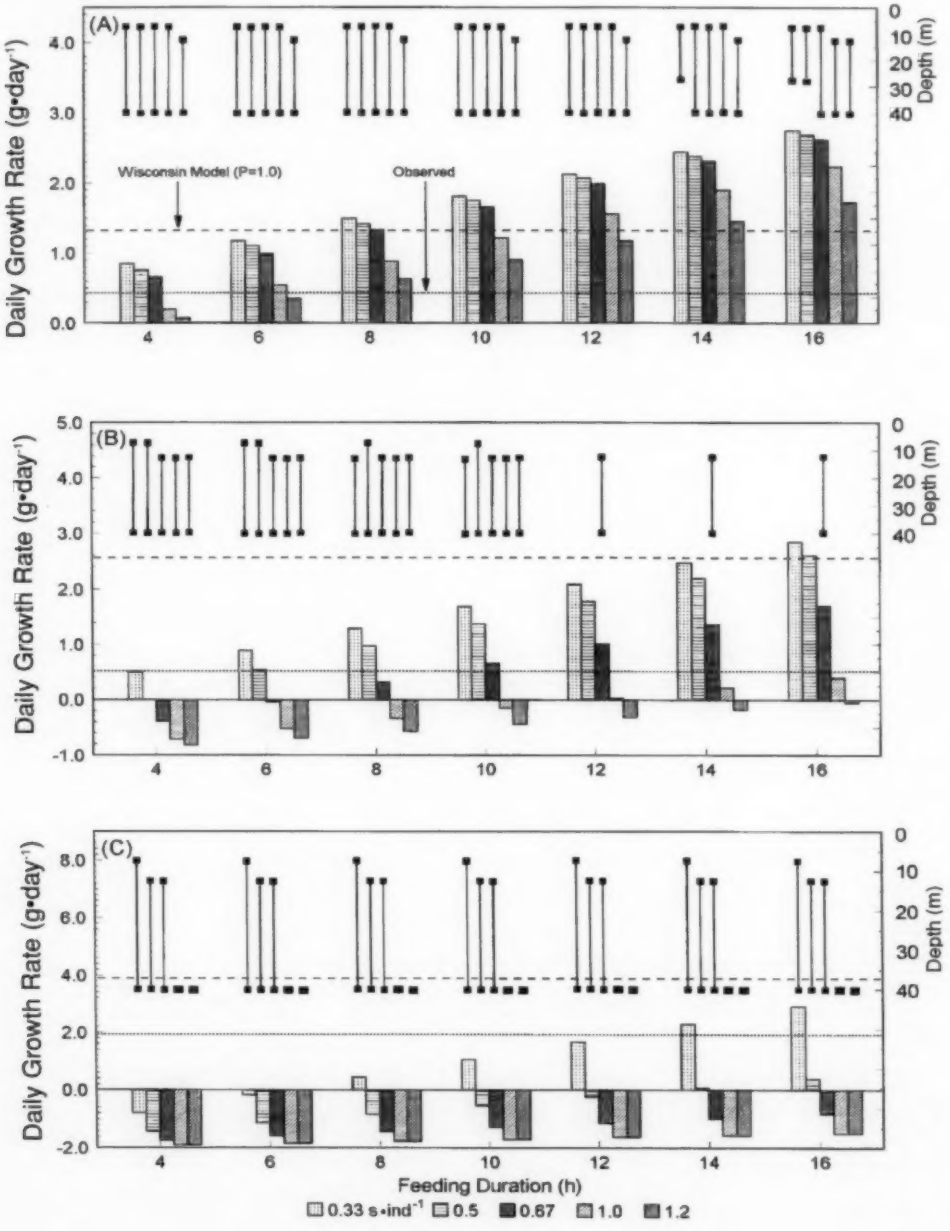
Fig. 8. Same as Fig. 7, but for limnological sampling date 18 August 1994. Starting kokanee masses for simulations were 121.15 g for age 1, 342.45 g for age 2, and 722.34 g for age 3.



Third, when allowed to feed for 16 h (midsummer day length) in the modified model, age-1 BMR kokanee were predicted to have maximum growth rates nearly double the maximum scope for growth estimate (at the optimum temperature) from the Wisconsin model (Figs. 6A, 7A, and 8A). In this case, only a small portion of the difference (~10%) can be attributed to the energetic efficiency of vertical migration; smaller fish are more tolerant of warmer water and therefore not as restricted in their upper thermal range compared with larger fish. Rather, the differences in predicted growth rates are a result of the differences in maximum consumption rate in each model.

The discrepancy points out the need to accurately extrapolate from laboratory feeding experiments to feeding behavior

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in the field. This is an important issue given the exciting technological advances in spatially explicit modeling of predator–prey interactions (Brandt et al. 1992; Mason and Patrick 1993; Mason et al. 1995). We suspect that feeding rate predictions differ because of different time scales used in each model. In the modified B&A model, fish feed in 30-min time steps. Maximum consumption over 24 h is determined by feeding duration, stomach capacity, and gastric evacuation rate. Additionally, the functional response was derived at a 1-min time scale with fasted fish, and therefore, motivation to feed is maximal in the model as long as there is any unfilled stomach volume. Because satiation does not enter directly into the calculation of in situ feeding rate, long feeding durations may predict unrealistic daily consumption rates in the modified model. However, Godin (1981) experimentally demonstrated that feeding rates of juvenile pink salmon (*O. gorbuscha*), after satiation, were approximately equal to their gastric evacuation rate. More work is needed to determine the importance of satiation on the functional response of age-1 and older kokanee.

In the Wisconsin model, maximum daily consumption rate was derived from feeding experiments where fish were fed excess rations three times a day (Brett et al. 1969; Brett 1971). It is not clear how scaling three distinct feeding bouts to 24 h might affect maximum daily consumption estimates, compared with a more continuous feeding regime typical of a pelagic planktivore. Furthermore, results of feeding experiments using pelleted food may not be applicable to fish in the wild (Beauchamp et al. 1989). Beauchamp et al. (1989) state that their model of maximum consumption should be considered an hypothesis. In light of our results, we hypothesize that maximum consumption rates of age-1 kokanee (<150 g) can be considerably higher than previously predicted by the Wisconsin model.

Alternatively, Luecke and Brandt (1993) found that rainbow trout increased the mass of *Daphnia* in their stomachs by reducing (squeezing out) the water content of their prey. This effectively doubled the energy density of the *Daphnia*. If kokanee are capable of this, then our model would overestimate the feeding durations and (or) underestimate the prey handling times required to bound observed growth. It would also explain why growth estimates from the Wisconsin model required a $P > 1.0$ to approach observed growth of age-1 and -2 kokanee in July. However, even doubling growth estimates from our modified model for age-3 kokanee in August would still require a feeding rate of 3 *Daphnia*·s^{-1} to approach observed growth.

Finally, the model predicted ontogenetic shifts in migration strategies at both seasonal and annual time scales, consistent with expectations from the bioenergetics principle that thermal optima are mass dependent. As the growing season progressed, age-1 fish in the modified model fed at greater depths and migrated longer distances. Age-2 and -3 fish showed similar but weaker patterns for the first half of the growing season. Across age-classes, the model demonstrated that older fish tend to feed at and migrate to greater depths at any given point in time.

For all simulations, we kept kokanee reaction distance constant (0.08 m) regardless of feeding depth. This is an unlikely assumption given the changes in light level with depth at both diurnal and seasonal time scales. Based on Secchi depths for Blue Mesa Reservoir on the three simulation dates (2.9, 3.9, and 5.6 m for June, July, and August, respectively; B.M. Johnson, unpublished data), calculated changes in percentage transmission of surface light from a depth of 2.5–12.5 m as a function of Secchi depth (Wetzel 1983), and measured reaction distances of several fish species over a range of light intensities (O'Brien 1979), we reduced kokanee reaction distance to 0.04 m and ran several simulations across the three simulation dates. Decreased reaction distance did not affect model growth rates of smaller kokanee because their smaller stomach capacities were saturated at the high prey densities. However, model growth rates of fish that were predicted to maximize growth by feeding at 12.5-m depth (age-2 kokanee in August, and age-3 model kokanee in July and August) were reduced two to five times. Consequently, the optimum migration strategies in these cases shifted to feeding depths of <10 m, where light levels and reaction distances would be presumably greater. However, growth rates would be lower than initial simulations (reaction distance 0.08 m) because of warmer temperatures at these feeding depths. This is particularly important to note for age-3 fish in August, where the gap between observed and model growth would widen, enforcing the notion that these fish might have to feed at a greater rate and (or) for longer periods of time. Future modifications to the functional response to include the effects of light levels on reaction distance would add another level of sophistication to the model, and might provide more insights into the foraging ecology of kokanee.

We have been sampling kokanee with both experimental vertical gill nets and sonar since 1993 to examine diets and to estimate population abundances. The sampling designs employed do not provide adequate temporal resolution and coverage to appropriately test our model predictions of migratory behavior. Other studies have found similar differences between day and night vertical distributions of kokanee (e.g., Finnell and Reed 1969; Maiolie and Elam 1996), although these patterns are not ubiquitous and may even be reversed (e.g., Chapman and Fortune 1963; Levy 1991). A comprehensive series of diel surveys in BMR is planned for the 1997 field season to corroborate model predictions.

The model predictions, while not fully tested in this study, demonstrate the predictive capabilities that are possible by adding more ecological realism to the bioenergetics framework. Despite enjoying wide use, application, and in a few cases corroboration (Hansen et al. 1993; Ney 1993), the Wisconsin model lacks some of this ecological realism. Ecosystem managers need predictive models to forecast individual responses and community and ecosystem consequences of natural and anthropogenic environmental change. In reservoirs, climate and water management interact to determine stratification patterns, with obvious implications for lentic biota. In response to the increasing demand on reservoir water in the western United States to serve an expanding range of purposes, traditional reservoir operation schedules are being changed. Large releases to simulate natural floods for downstream habitat restoration (Wuethrich 1996), periodic releases to help transport salmon smolts to the ocean (Berggren and Filardo 1993), and spring releases to mimic a natural snowmelt hydrograph and improve spawning conditions for endangered Colorado River fishes (Tyus 1992) all are significant departures from historic operations. Few, if any studies have

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addressed the upstream (reservoir) effects of these new operation strategies.

Our intent in further developing the B&A approach was to generate a modeling framework that was sufficiently realistic and accurate to predict effects of seasonal variation in vertical gradients of temperature and prey density on kokanee growth, behavioral response, and population consumption demand. It is reasonable to hypothesize that changing reservoir operations could alter temperature and prey stratification, imposing constraints on kokanee depth distribution, especially in reservoirs such as BMR that are near the upper thermal limit for kokanee. Consequences might include reduced access to predominately epilimnial zooplankton resources, reduced scope for growth, and increased spatial and temporal overlap with, and hence, predation risk from the primary piscine predator, the lake trout (e.g., Martinez and Wiltzius 1995). The modeling framework further developed here provides the means to predict the effects of reservoir stratification scenarios on fish behavior and growth, and as such, could be a powerful tool for understanding the ecological effects of anthropogenic environmental change.

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Captive gummy sharks, *Mustelus antarcticus*, form hypermineralised bands in their vertebrae during winter

Rickard A. Officer, Rob W. Day, John G. Clement, and Lauren P. Brown

Abstract: The subjective interpretation of the growth increments in shark vertebrae and uncertainty over the temporal periodicity of their formation decrease confidence in the accuracy of ages estimated from increment counts. To determine the periodicity with which growth increments are formed in the vertebrae of gummy shark, *Mustelus antarcticus*, fluorochrome dyes were injected into captive sharks at quarterly intervals. Variation in the vertebral growth rate between fluorochrome labels was examined and compared with measurements of the density of mineralisation of vertebral sections. The slowest rates of vertebral growth and the highest radiographic densities were found in the vertebral tissues formed during winter. The microradiographic method described here resolves individual hypermineralised increments that are indistinguishable using surface reading methods. With this extra information, a reader may better count, group, or exclude certain growth increment patterns when making an age estimation. Counts of clusters of hypermineralised increments may be more useful in age determination than counts of individual increments because individual increments form unpredictably and possibly result from brief, irregular episodes of slow vertebral growth.

Résumé : La précision des estimations de l'âge des requins par dénombrement des stries de croissance dans les vertèbres est amoindrie par l'interprétation subjective de ces accroissements, ainsi que par l'incertitude concernant la périodicité temporelle de leur formation. Afin de déterminer la périodicité de la formation des stries de croissance dans les vertèbres d'émissolles gommées, *Mustelus antarcticus*, nous avons injecté tous les trimestres des teintures au fluorochrome à des requins en captivité. La variation du taux de croissance des vertèbres entre les marqueurs au fluorochrome a été calculée et comparée aux mesures de la densité de la minéralisation des sections de vertèbre. Les résultats ont révélé des taux de croissance les plus lents et des densités radiographiques les plus élevées dans les tissus qui se sont formés en hiver. La méthode microradiographique décrite ici permet de distinguer des stries de croissance hyperminéralisées individuelles qui ne peuvent l'être au moyen de méthodes de lecture en surface. Ce supplément d'information peut permettre de mieux dénombrer les stries ou d'en regrouper ou d'en exclure certaines pour estimer l'âge. Il peut être plus utile de dénombrer des regroupements de stries de croissance hyperminéralisées pour déterminer l'âge des individus que de dénombrer les stries elles-mêmes, parce que celles-ci se forment de façon imprévisible et qu'elles résultent parfois de brefs épisodes irréguliers de lente croissance des vertèbres.
[Traduit par la Rédaction]

Introduction

In previous studies, it has been demonstrated that fine, minor vertebral growth increments may form at all times of year in captive gummy sharks, *Mustelus antarcticus* (Officer 1995), and that cellular activity at the margins of vertebrae almost certainly modulates the production of discrete hypermineralised increments (Clement 1992; Officer 1995). The results of these studies suggest that the process of vertebral growth in captive sharks is normally punctuated by episodes of altered cellular activity and that narrow bands of hypermineralised tissue in the vertebrae may represent the brief slowing of vertebral growth during these episodes.

Such minor growth increments may therefore not always form periodically and may confuse a reader attempting to determine the age of a shark from a count of all its vertebral growth increments. Any periodic variations in the mineralisation pattern in the vertebrae may be obscured by a superimposed pattern of increments resulting from brief episodes of growth.

Other authors have shown that seasonal changes in the vertebral growth rate are accompanied by seasonal changes in the degree of vertebral mineralisation (Ishiyama 1951; Holden and Vince 1973; Stevens 1975; Tanaka et al. 1978; Cailliet et al. 1983; Cailliet and Radtke 1987; Ferreira and Vooren 1991). Similarly, in gummy sharks, chondrocytes exhibiting the features characteristic of slow cellular growth and tissue accretion rates were associated with narrow bands of hypermineralised tissue (Officer 1995). Thus, longer periods of slow growth might result in broader bands of hypermineralisation. Prolonged periods of slow growth are likely to be seasonal because factors that limit growth capacity, such as food availability and water temperature, are themselves seasonal (Jones and Geen 1977; Kusher et al. 1992).

In the present study, fluorochrome dyes were injected at

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Table 1. Dates of injection and measurements of each of the gummy sharks that shared the year in captivity.

Shark No.	Sex	First injection Date	TL (mm)	TW (kg)	CR (mm)	Second injection, June 1, 1993 TL (mm)	TW (kg)	CR (mm)	Third injection, Sept. 7, 1993 TL (mm)	TW (kg)	CR (mm)	Fourth injection, Dec. 21, 1993 TL (mm)	TW (kg)	CR (mm)	Final injection/death, Mar. 17, 1994 TL (mm)	TW (kg)	CR (mm)
16	M	Feb. 23, 1993	1190	7.1	6.64	1230	7.9	6.83	1230	8.0	6.90	1220	7.8	6.97	1240	8.3	7.06
17	M	Feb. 23, 1993	1070	5.3	5.74	1070	5.4	6.02	1090	5.6	6.09	1100	5.7	6.25	1120	6.1	6.39
20	M	Feb. 23, 1993	1060	4.9	5.92	1090	6.1	6.11	1100	6.1	6.18	1110	6.4	6.31	1130	6.7	6.42
36	M	Feb. 23, 1993	830	2.4	4.15	940	3.6	4.75	980	3.8	4.94	1040	4.6	5.29	1090	5.3	5.55
40	M	Mar. 19, 1993	760	1.7	3.84	820	2.5	4.19	840	2.4	4.33	860	2.8	4.49	930	3.4	4.86
41	F	Mar. 19, 1993	780	2.0	4.11	830	2.4	4.47	830	2.3	4.52	830	2.2	4.57	910	3.4	4.98
42	M	Mar. 19, 1993	1010	4.2	5.43	1020	4.3	5.64	1030	4.3	5.67	1050	4.9	5.85	1090	5.7	6.10
45	M	Mar. 19, 1993	1180	6.2	6.41	1180	6.8	6.54	1190	7.5	6.59	1200	7.6	6.65	1210	7.4	6.70
51	M	Apr. 1, 1993	600	0.6	2.75	650	1.2	3.03	690	1.5	3.28	780	2.1	3.73	860	2.8	4.28
53	F	Apr. 22, 1993	1030	4.8	5.30	1030	4.8	5.35	1040	5.3	5.40	1100	6.7	5.78	1160	8.5	6.18

Note: TL, total length (mm); TW, total weight (kg); CR, centrum radius (mm) measured from the focus to fluorescent bands or, at death, to the vertebral margin.

quarterly intervals into captive gummy sharks. The resulting delimitation of the vertebral tissues formed during each season provided a means of comparing between seasons the rate of vertebral growth and degree of vertebral mineralisation achieved by individual sharks.

Materials and methods

Thirty-three gummy sharks were collected between January 1992 and May 1994 from southern Australian coastal waters and transferred to the Marine and Freshwater Resources Institute (MAFRI) where they were held in captivity. The total lengths and weights of the sharks were measured, and passive integrated transponder tags (ID-100, Central Animal Records P/L, Victoria, Australia) were implanted for individual identification. The total length of each shark was measured to the nearest millimetre after the tail had been allowed to take a natural position and after the top caudal lobe had been placed parallel to the body axis.

Prior to injection with fluorochrome marking dyes, the sharks were anaesthetised by immersion in a bath of MS 222 anaesthetic (50 mg/L seawater) (Sigma Aldrich P/L, New South Wales, Australia). Doses of fluorochrome dyes were calculated as a proportion of the shark's total body weight. The selected fluorochrome dye was injected intraperitoneally. Following injection the sharks were temporarily transferred to a 15 000-L aquarium to monitor their recovery. The sharks were then kept in a 27 000-L outdoor aquarium (Aqualok Aquaculture tank (AFT6), Seafood Technologies P/L, Victoria, Australia). The stocking density never exceeded 1 kg of shark per 350 L of seawater. A continuous supply (>10 000 L/h) of fresh seawater and the outdoor location of the aquarium meant that the captive sharks were subject to natural fluctuations in water temperature, quality, and photoperiod. Aquarium temperatures were consistent with sea-surface temperatures measured within 8 nautical miles of the MAFRI. The sharks were fed ad libitum on a diet of squid, pilchards, prawns, and mussels similar to the diet normally consumed in the wild (Coleman and Mobley 1984). The diet was supplemented with vitamins and minerals recommended for use in captive elasmobranchs (Murru 1990).

Oxytetracycline hydrochloride (Terramycin, Pfizer Agricare P/L, Victoria, Australia) was used because of its reported ease of use and consistency of results in other species of elasmobranchs (Holden and Vince 1973; Smith 1984; Tucker 1985; Branstetter and McEachran 1986; Clement 1986; Tanaka 1990; Kusher et al. 1992). However, the need to reinject the sharks every quarter meant that oxytetracycline was not injected repeatedly. To avoid the possibility of not being able to attribute a particular oxytetracycline label to a particular injection due to failure in marking (Tanaka 1990; Kusher et al. 1992), alizarin red S, alizarin complexone, calcein, and xylenol orange were also used. These dyes have an affinity for mineralising tissues similar to that of oxytetracycline, but can be distinguished because they fluoresce at different wavelengths.

Alizarin red S and calcein derivatives have been used in gastropods (Sire and Bonnet 1984), common carp, *Cyprinus carpio* (Yamada 1971), carangids (Trebaol et al. 1991), and reptiles (Kariyama et al. 1969), xylenol orange has been used in carangids (Trebaol et al. 1991) and nutrias (Pascal et al. 1989), and alizarin complexone has been used in salmonids (Tsukamoto 1988). Clement (1986) used a calcein derivative in elasmobranchs. We are not aware of other studies of elasmobranchs that have utilised alizarin red S, alizarin complexone, calcein, or xylenol orange to mark the internal structure of vertebrae.

Following the recommendations of other authors, a standard dose of 25 mg/kg total weight was adopted for oxytetracycline (McFarlane and Beamish 1987), calcein (Suzuki and Mathews 1966), and alizarin red S (Adkins 1965). Alizarin complexone doses were also 25 mg/kg, as the concentrations of alizarin complexone solutions used for immersion marking by Tsukamoto (1988) were of the same order of magnitude as those of the other dyes. Xylenol orange was initially injected at a dose of 90 mg/kg (Rahn and Perren 1971; Trebaol et al. 1991). A lower dose of 50 mg/kg was used in later injections because such doses are still able to induce marks but with less possibility of ill effect on the sharks. Alizarin red S, alizarin complexone, calcein, and xylenol orange (Sigma Aldrich P/L, New South Wales, Australia) were dissolved in 1 M saline solutions of sodium bicarbonate and injected at a concentration of 100 mg/mL solution.

Ten gummy sharks that had been injected with the fluorochrome dyes and held in captivity for 1 year (Table 1) were sacrificed by cutting the spinal cord immediately behind the head. Two vertebrae were dissected and examined from each shark. These were (1) the first complete immediately postcranial vertebra and (2) one of the largest vertebrae in the vertebral column, which was selected from the trunk region posterior to the first dorsal fin. Immediately postcranial vertebrae were selected, as these vertebrae are used for routine age determinations to monitor the southern Australian gummy shark fishery (Walker and Moulton 1992). We anticipated that the growth of larger vertebrae would be greater than that of smaller vertebrae. The consequent greater separation of growth increments in trunk vertebrae would allow measurement of vertebral growth in cases where increments were not sufficiently resolvable in smaller vertebrae. Furthermore, other studies have suggested that trunk vertebrae have a greater readability than immediately postcranial vertebrae (Officer 1995; Officer et al. 1996). The beginning and end of each seasonal growth interval were delineated by the position of the fluorochromes injected, so that variations in growth rate could be examined.

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Longitudinal sections 100 µm (±10 µm) thick were cut through the focus and lateral intermedialia of each vertebra using a Leitz 1600 sawing microtome (Leica Instruments P/L, Victoria, Australia). Sections were stored in 70% ethyl alcohol in the dark to prevent degradation of the dyes and any corresponding loss of fluorescence.

The spacing between differently coloured fluorochrome labels in vertebral sections was measured by a computer-linked fluorescence microscope system based on a design by Dykes and Clement (1980). This system was used because it was extremely accurate (Dykes et al. 1981) and overcame the problems of distortion and insufficient light intensity common to other methods of examination of fluorochrome dyes.

The microscope system was used to digitise the *X*- and *Y*-coordinates of the focus of the section, and the proximal side of each fluorochrome label and the vertebral margin were digitised at the points where each was the maximum radius from the focus of the section. As fluorochrome dyes are incorporated rapidly into mineralised tissues (Milch et al. 1957) but persist in the circulation for some time (Clement 1986), the proximal side of each label was assumed to be the site of mineralised tissue deposition on the date of injection. The microscope system was calibrated and Pythagoras' theorem was used to calculate the distances from the focus to each fluorochrome label and the vertebral margin. An average was calculated from three sets of measurements made using a variety of light filtering combinations appropriate to the dyes present in the section. All distances were expressed as a proportion of the centrum radius so as to compare label positions between vertebrae. Vertebral growth rates were expressed in micrometres per day and represented the average linear growth rate for the time interval between repeated injections or between the last injection and the shark's death.

The same sections were used for both fluorescence microscopy and microradiographic examination of mineralisation patterns. Microradiographs were made using high-resolution microradiographic equipment (OEG tube, Machlett Laboratories Inc., Stanford, Conn.) and high-resolution glass plates (type 1A, Eastman Kodak Co., New York). The vertebral sections had to be microradiographed wet because drying would distort the original dimensions of the sections. An impermeable plastic film, 40 µm thick, was used to separate the vertebral sections from the emulsion and to protect the emulsion from the alcohol. The glass plates were positioned 16.5 cm from the focus of the X-ray tube and exposed at 20 kV, 10 mA, for 18 min. The plates were developed according to the manufacturer's directions.

Microradiograph images were scanned using a high-resolution scanner (ScanMaker MTS 1850, Microtek International Inc., Hinschu, Taiwan). The use of light from a point source was advantageous because all parts of the microradiographs were scanned under identical illumination. An image analysis program (Optimas version 4.1.1, BioScan Inc., Bothell, Wash.) measured the optical density in transects across each image along the same plane as the fluorochrome measurement to allow comparisons.

Five parallel transects were drawn across the image of the heavily mineralised corpus calcareum of each section, from the focus to the vertebral margin. The computer program measured the grey level of the pixels on an arbitrary 256-unit scale at 250 equally spaced points along each transect. The scale measured the optical density of the microradiograph (Martin et al. 1990). Completely radiolucent points appeared black and were assigned a grey level of 1. More radiopaque points were assigned higher scores up to a maximum of 256 (pure white).

The position of each fluorochrome label along the transects that delineated the beginning and end of each growth interval was found by multiplying their relative position by 250, the number of points in each of the transects. The average grey level for each growth interval was calculated from all five transects.

Because sections unavoidably vary slightly in thickness and thus overall grey levels, the relative differences in the degree of mineralisation, rather than the absolute mineral density, were compared between seasons. The relative degree of mineralisation during each growth interval was calculated as the natural logarithm of the average grey level, following Martin et al. (1990) and Squillante and Williams (1993). The relative mineral density (RMD) of the section during each season was expressed as a percentage of the average mineral density for the year using the equation

$$\mathrm{RMD} = \left(\frac{\ln G_{\mathrm{season}}}{(\ln G_{\mathrm{autumn}} + \ln G_{\mathrm{winter}} + \ln G_{\mathrm{spring}} + \ln G_{\mathrm{summer}})/4} \right) \times 100$$

where ln G is the natural logarithm of the average grey level for each season. These values were compared within and between vertebral sections.

Data analysis

Variation in vertebral growth rate and RMD were analysed using univariate analyses of variance with repeated measures. The following model was used:

$$\text{Vertebral growth rate or RMD} = \text{Region} + \text{Season} + \text{Region} \times \text{Season} + \text{Shark} + \text{Error}$$

Region was included in the analysis to determine if relative changes in growth rate occur between regions with season. The mean vertebral growth rate and RMD for each region of the vertebral column during each season was also calculated.

Results

Newly incorporated fluorochrome dyes were only visible at the very margin of the vertebrae. Fluorochrome labels resulting from successive injections were always deposited superficially to previously incorporated dyes (Fig. 1), indicating that vertebral growth occurred strictly by apposition, that growth of the vertebrae was continuous, and that accretion of new mineral occurred at all times of the year. The location and rapid incorporation indicated that fluorochrome labels accurately defined the growing margin of the vertebrae at the time of injection.

Fine, discrete bands of hypermineralised tissue were formed throughout the entire year (Fig. 1), possibly as a result of disturbance following handling. Incorporated dyes occupied relatively narrow, discrete bands (Fig. 1). Although injected at regular intervals, the fluorochrome labels had an irregular spacing (Fig. 1), which indicated that the growth of the vertebrae varied. In most cases, fluorochrome labels were well separated by unmarked mineralised tissue, suggesting that the dyes remained for a relatively short period in the shark's circulation at sufficient concentration to label mineralising tissues.

Growth rates were highly variable between individuals and between seasons. The lower vertebral growth rates found during winter were consistent with the seasonal variation in water temperature (Fig. 2). Significant differences in vertebral growth rate were detected between regions of the vertebral column ($F_{1,63} = 39.05$, $P < 0.01$) and between seasons ($F_{3,63} = 26.65$, $P < 0.01$) (Table 2). As the sharks were of mixed sexes and differing sizes (Table 1), the significant differences in growth rate detected between sharks were expected ($F_{9,63} = 10.46$, $P < 0.01$) (Table 2). No significant interaction between the region of the vertebral column and season was detected (Table 2). The mean vertebral growth rates in both regions of the vertebral column were least during the winter growth interval and greatest during the autumn growth interval (Table 3; Fig. 3).

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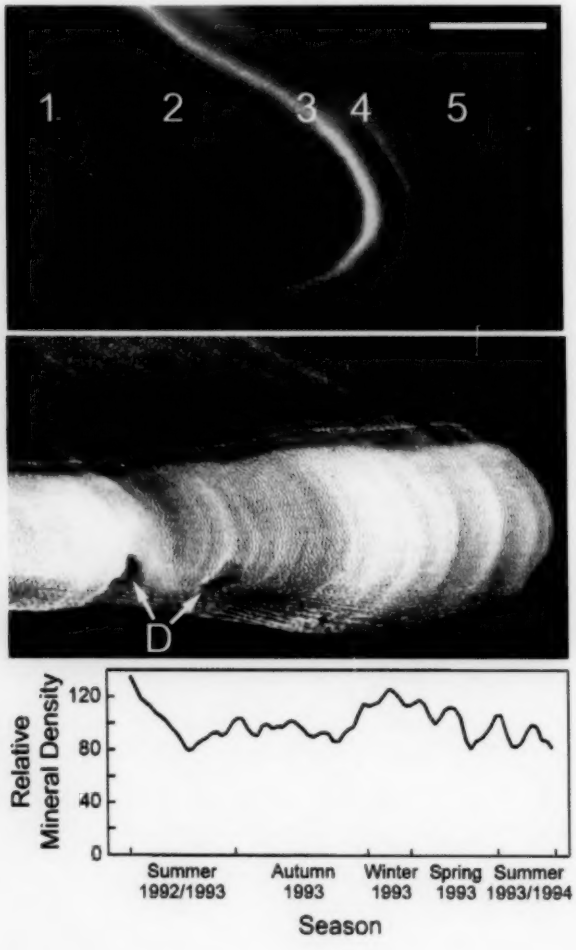
Fig. 1. Fluorescence photomicrograph (top), microradiograph (middle), and greyscale trajectory (bottom) of the margin of a vertebral section from a captive gummy shark. Despite being injected at regular intervals on five separate occasions, the dyes were unevenly spaced and indicated that the vertebral growth rate was least during winter. Minor growth increments were formed all year, but the vertebral tissues formed during winter displayed the highest degree of mineralisation. Hypermineralised increments that probably formed following handling are indicated (D). Injection details (lengths are total lengths): (1) December 9, 1992, alizarin complexone, 750 mm; (2) February 23, 1993, alizarin complexone, 830 mm; (3) June 1, 1993, calcein, 940 mm; (4) September 7, 1993, oxytetracycline, 980 mm; (5) December 21, 1993, xylenol orange, 1040 mm; (death): March 17, 1994, 1090 mm. Scale bar = 0.5 mm.



Significant differences in RMD were only detected between seasons ($F_{3,63} = 18.51$, $P < 0.01$) (Table 2). The mean RMD values were highest during the winter growth interval for both postcranial and trunk vertebrae (Table 3; Fig. 3). The least mineralised vertebral tissues were formed during the summer growth interval (Table 3; Fig. 3). Higher mean RMDs were found during winter (Table 3; Fig. 3).

Fig. 2. Water temperatures in the aquarium and in Port Phillip Bay during the course of the experiment and the average seasonal growth rates of immediately postcranial vertebrae from 10 gummy sharks that shared the same year in captivity.

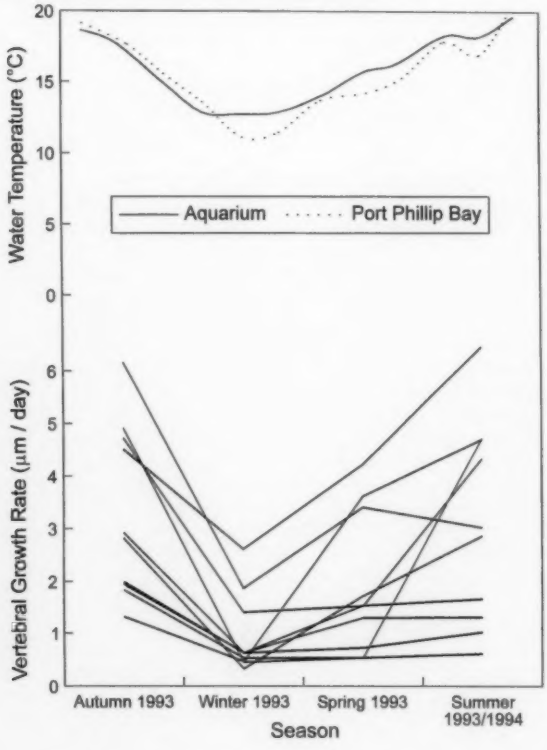


Table 2. Results of repeated measures analyses of variance in the growth rates of gummy shark vertebrae and the relative mineral densities of gummy shark vertebral sections (results are significant at $P < 0.05$).

Source	Degrees of freedom	Mean square	F-ratio	P
Growth rate				
Region	1	58.68	39.05	<0.01
Season	3	40.06	26.65	<0.01
Region × season	3	3.02	3.01	0.122
Shark	9	15.71	10.46	<0.01
Error	63	1.50		
Relative mineral density				
Region	1	0.47	0.13	0.719
Season	3	67.03	18.51	<0.01
Region × season	3	6.14	1.69	0.177
Shark	9	3.77	1.04	0.418
Error	63	3.62		

Discussion

Hypermineralisation of the vertebral tissues formed during winter has been described in several other genera of elasmobranchs (Ishiyama 1951; Tanaka et al. 1978; Killam and Parsons 1989; Ferreira and Vooren 1991; Parsons 1993).

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Table 3. Seasonal means of the growth rates (μm/day) of gummy shark vertebrae and the relative mineral densities of gummy shark vertebral sections.

Region of column	*n*	Autumn 1993 Mean	Autumn 1993 SD	Winter 1993 Mean	Winter 1993 SD	Spring 1993 Mean	Spring 1993 SD	Summer 1993–1994 Mean	Summer 1993–1994 SD
Growth rate									
Postcranial	10	3.3	1.6	1.0	0.8	1.9	1.3	3.1	1.9
Trunk	10	6.1	2.3	2.2	1.2	2.9	1.9	4.8	2.7
Relative mineral density									
Postcranial	10	101.2	1.6	102.5	2.0	101.0	1.5	97.7	1.4
Trunk	10	99.8	1.8	102.7	1.2	101.7	2.9	98.8	2.3

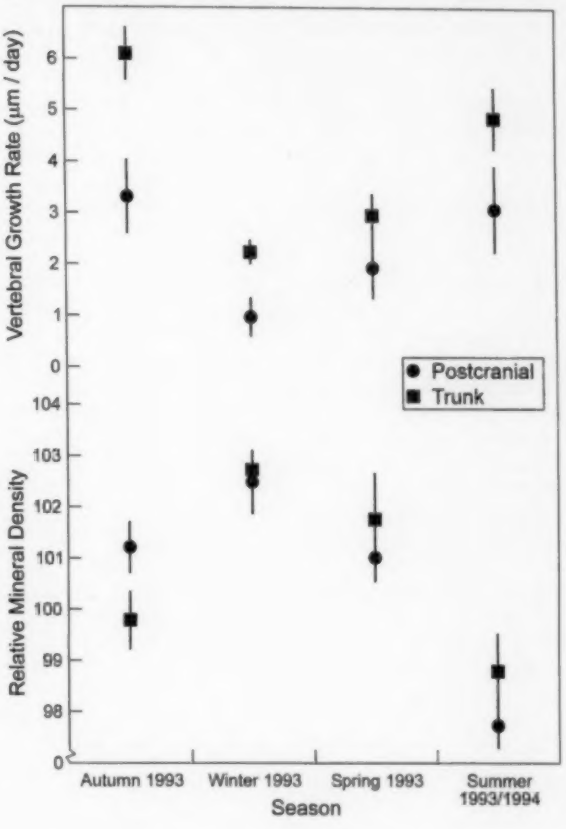
Although only a small number of gummy sharks were available for this study, the results suggest that in gummy sharks the vertebral tissues formed during the winter months are hypermineralised.

Hypermineralisation was also related to periods of slow growth. This result is consistent with previous studies of elasmobranchs (Ishiyama 1951; Jones and Geen 1977; Tanaka et al. 1978; Killam and Parsons 1989; Ferreira and Vooren 1991; Parsons 1993). Jones and Geen (1977) suggested that heavier mineralisation in winter in the spiny dogfish, *Squalus acanthias*, could be explained by the relative abundance of phosphorus during winter. Phosphorus is a necessary ingredient of hydroxyapatite, the principal component of the mineral phase of elasmobranch mineralised cartilage matrix (Urist 1961; Moss 1977). The secretion of alkaline phosphatase is required to precipitate mineral salts into the extracellular matrix that surrounds each chondrocyte and eventually cement these cells into the mineralised vertebral centrum to facilitate appositional growth (Bancroft and Stevens 1990). Temporary increases in the abundance of phosphorus could enhance an elasmobranch's capacity to mineralise vertebral tissues.

Electron microprobe analyses of the vertebrae from the grey reef shark, *Carcharhinus amblyrhynchos*, and thresher shark, *Alopias vulpinus* (Cailliet and Radtke 1987), showed that less calcium and phosphorus were deposited during the winter than during the summer. This suggests that the vertebral tissues formed in these sharks during summer were more heavily mineralised. However, the winter growth zones in the vertebrae of these sharks were translucent to transmitted light (Cailliet and Radtke 1987), a characteristic of hypermineralised zones in the vertebrae of other elasmobranch species (Ishiyama 1951; Kusakari 1969; Jones and Geen 1977; Tanaka et al. 1978; Killam and Parsons 1989; Ferreira and Vooren 1991; Parsons 1993) and other sclerotic skeletal tissues such as dentine and dental enamel (Moss-Salentijn and Hendricks-Klyvert 1985; Bhaskar 1991). Cailliet and Radtke's (1987) finding that vertebral regions with the optical features characteristic of hypermineralised tissue apparently contained lower levels of calcium and phosphorus contradicts the results of the present study. In grey reef and thresher sharks, increased phosphorus availability in winter is perhaps unrelated or only indirectly related to the increased mineral density in vertebral growth zones that form during winter.

In some teleost fish, the amount of protein deposited in the developing otolith is considered to be an important determinant of the differences in mineral density observed in otoliths

Fig. 3. Average seasonal growth rates and relative mineral density of postcranial and trunk vertebrae from 10 gummy sharks that shared the same year in captivity. Bars indicate standard error.



(Mugiya 1965). Mugiya's results suggest that the amount of soluble protein taken into the mineralising otolith and deposited in the organic matrix in an insoluble form may vary seasonally, with the concentration of circulating soluble protein. We are not aware of any studies to determine if a similar mechanism operates in elasmobranch vertebrae.

While elucidating which factors influence vertebral hypermineralisation is an important area yet to be studied fully, our incomplete understanding does not preclude interpreting hypermineralisation patterns as a record of the seasonal changes in an elasmobranch and possibly using these patterns to estimate age. The distinct winter hypermineralisation of vertebral growth zones in the gummy shark vertebrae examined in the present study suggested that the interpretation of minor growth increments as periodically formed marks may be inappropriate. The hypermineralised regions resulting from prolonged periods of slow growth were displayed in microradiographs as broad radiopaque bands, or as unresolved clusters of narrow, hypermineralised increments, each the probable product of a separate episode of disturbance. Back-calculation of the dates of formation of such narrow increments in captive gummy sharks injected with fluorochrome dyes (Officer 1995) indicated that some sharks formed several heavily mineralised increments during the course of a year and that several

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hypermineralised increments could be formed each only a few weeks apart. In the present study, the most prolonged periods of slow growth occurred during winter. Hence, counts of clustered increments appear to represent a winter growth period and these clusters often appear as a broad hypermineralised band.

More major and minor hypermineralised vertebral growth increments were observed in the vertebrae of sharks kept in captivity during the course of these experiments than in the vertebrae from returned tagged and similarly labelled wild sharks (Officer 1995). Deposition of minor nonperiodic growth increments is possibly not as common in wild sharks as it is in captive sharks because the growth of wild sharks may not be disturbed as often, or as profoundly, as that of captive sharks. Minor increments have been reported in the vertebrae of other species of elasmobranch caught in the wild (Cailliet and Radtke 1987). Such increments in wild sharks may confuse an inexperienced reader attempting to estimate age from interpretation of a vertebral section. However, because minor growth increments may form less often in wild sharks, the difficulties in distinguishing between major and minor growth increments may be less problematic when examining vertebrae from wild sharks.

The alizarin staining method currently in use in the routine ageing of vertebrae in Australia's southern shark fishery is unable to resolve fine increments (Moulton et al. 1992). However, counts of bands made using surface reading methods that are unable to resolve clustered minor increments may successfully estimate the age of a shark because clusters of minor increments are usually interpreted as single broad zones of seasonally hypermineralised tissue (Officer et al. 1996). This consolidation of increments may explain the reasonable agreement between von Bertalanffy growth curves produced from tag length-increment data and curves produced from increment counts of stained whole vertebrae (Moulton et al. 1992). The agreement between these growth curves provides further evidence that major hypermineralised bands are formed annually in gummy sharks. Despite this apparent verification of the methods being used, validation of the age estimates they provide still requires an understanding of the temporal periodicity of growth increment deposition in the wild.

The better resolution of the microradiographic method used in this study makes fine vertebral increment patterns readily visible. Such increments are indistinguishable using the alizarin staining method. Counting all of the fine increments seen on microradiographs of vertebral sections without an understanding of how and when they are formed might produce an inflated age estimate for a shark. However, a trained reader could subjectively exclude minor increments and count only the major bands of hypermineralised tissue which more probably represent winter hypermineralisation. The microradiographic method may also allow more accurate estimation of the age of vertebrae from large sharks. These sharks are difficult to age using the alizarin staining method (Moulton et al. 1992) because the marginal increments are very close together, reflecting the slow growth of older sharks. The increments visible in large shark vertebrae using the microradiographic method are distinct even at the outer margins of the vertebrae, the region most difficult to interpret using surface reading methods (Holden and Vince 1973).

The results of this study indicate that bands of relatively dense tissue probably form during winter in gummy shark vertebrae. This result is of direct use in age determination, as it suggests that growth band formation in gummy shark vertebrae may have a regular periodicity. The results of the study also suggest that the microradiographic method may be the best method of examining and counting growth bands in gummy shark vertebrae. While surface reading methods may fortuitously overcome their poor resolution by consolidating clusters of adjacent minor increments into broad bands of stained tissue, the microradiographic method also allows for such a subjective clustering of increments. Furthermore, the ability of the microradiographic method to resolve individual growth increments suggests that the method may be particularly useful in ageing sharks whose outermost vertebral growth increments are so closely apposed that they are indistinguishable using surface reading methods.

While these findings may have application to other elasmobranch species whose vertebrae are continuously mineralised, an understanding of the temporal periodicity of growth increment formation in wild sharks remains the only means of achieving age validation. Experiments such as this one are useful in the interim, as they may suggest the most likely pattern of growth increment formation long before the results of age validation studies are available.

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Variability of family size and marine survival in pink salmon (*Oncorhynchus gorbuscha*) has implications for conservation biology and human use

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Abstract: The short-term dynamics of salmonid populations are directly related to the mean sizes of individual families. The amount of genetic variation maintained in the population is directly related to the variance in sizes of individual families. Both the mean and variance of individual family sizes have important implications for conservation actions and sustainable levels of harvest of salmonid fishes. We develop a context for examining variation in family size, and we provide estimates of mean and variance of family size from five groups of marked pink salmon (*Oncorhynchus gorbuscha*) released into the north Pacific Ocean. We then present two important results: (1) a statistically detectable genetic component of marine survival exists in groups with high marine survival and (2) ratios of variance-to-mean family size are linearly related to mean family size over the interval that we observed. These results imply that short-term population increases come from a small fraction of the population's families, that salmon encounter a fluctuating marine environment, and that the most favored phenotype changes from generation to generation. These results also support the widely held view that protecting genetic variation in recovering or exploited salmon populations has important economic benefits.

Résumé : La dynamique à court terme des populations de salmonidés est directement liée aux tailles moyennes des familles qui les composent. L'ampleur de la variation génétique que présente une population est directement liée à la variance des tailles des familles de celle-ci. Tant la moyenne que la variance des tailles des familles ont d'importantes implications pour les mesures de conservation et les niveaux durables d'exploitation des salmonidés. Nous avons élaboré un cadre d'examen des variations de la taille des familles et fourni des estimations de la moyenne et de la variance de la taille des familles pour cinq groupes de saumons roses (*Oncorhynchus gorbuscha)* marqués et libérés dans le Pacifique Nord. Nous présentons ensuite deux conclusions importantes : (1) il existe une composante génétique de survie marine, statistiquement décelable, chez les groupes présentant un niveau élevé de survie en mer et (2) les rapports entre la variance et la moyenne de la taille des familles présentent une relation linéaire avec la taille moyenne des familles sur l'intervalle étudié ici. D'après ces résultats, il semble que les augmentations à court terme de la population soient liées à une faible fraction des familles de la population, que le saumon est exposé à des fluctuations du milieu marin et que le phénotype le plus favorable varie d'une génération à l'autre. Ces résultats confirment aussi l'opinion largement répandue selon laquelle le fait de protéger la variation génétique dans les populations en cours de rétablissement ou exploitées comporte des avantages économiques certains.
[Traduit par la Rédaction]

Introduction

The prevailing view in fisheries science is that the most important population parameter is abundance. Fisheries management and harvest decisions are based on abundance (Ricker 1975; Walters and Collie 1988), as are models used by conservation biologists in the analysis of the risk of extinction of populations (Ginzburg et al. 1982; Ferson et al. 1989; Dennis et al. 1991). For family size defined as the number of returning adult breeders per number of parents, abundance is just the product of family size and the number of breeders in the previous generation. In other words, abundance is equivalent to average family size for fixed breeding numbers. Little attention has been directed toward how family size varies in fish populations or how this variation indexes the amount of genetic variation that can be carried by a population. Although this variability has not been used explicitly in models of population dynamics or risk assessment, its importance has been acknowledged in conceptual models (Gilpin and Soulé 1986; Kapuscinski and Lannan 1986). Below, we present a context

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for relating family-specific survival variation at different life history stanzas to the genetic concept of effective population size, and we present an example where the population increased largely by increasing the variance of family size, not by a uniform increase in family size across most families.

At the extremes, either variation in anadromous salmon family size reflects only random influences or this variation reflects differing genetic approaches to survival in the marine environment. To test the hypothesis that family size is random, and not related to genetic information passed on from parents, we directly observed the number of pink salmon (*Oncorhynchus gorbuscha*) offspring, marked and tagged by family, surviving to return to their natal site in Southeast Alaska. By following the fate of families with more than one dam (female parent) per sire (male parent), we were able to use standard analysis of variance techniques to test for a genetic (sire) component to survival and family size (Falconer 1989). If half-siblings with the same sire but different dams have survival and family size that are more alike than fish with different dams and sires, then we infer that survival and family size are genetically influenced. To the degree that survival tended to be more similar between half-sibling groups with the same sire than among family groups with different parents, we interpreted this as evidence of genetic influence of average family size and survival.

Theoretical background

Our notation describes the size of populations and the properties of the families of individuals in those populations. Let N_t denote the number of adult breeders in a spawning population of pink salmon in generation t. Let k denote the random variable that describes the number of adult spawners produced in some family in generation t, and let $\bar{k}$ and $v(k)$ denote the mean and variance of family size. In our notation, $v(\ )$ denotes the observed variance among the families actually present and $V(\ )$ denotes variance with respect to some probability distribution.

Crow and Morton (1955) called the ratio $R_k = v(k)/\bar{k}$ the index of variability and described its calculation from adult progeny, from egg production, and from sampling variance in gene frequency. They showed that under random survival the index of variability tends to 1. In populations where the entire family survives or not as a unit, they showed that this ratio tends to $\bar{k}_{early} + v(\bar{k}_{early})/\bar{k}_{early}$ where "early" denotes a life stage occurring before the family-specific mortality. Even though values outside these limits are possible, the value of 1 for random survival and the value given by $\bar{k}_{early} + v(\bar{k}_{early})/\bar{k}_{early}$ for complete familial survival or mortality provide theoretical lower and upper reference values on the index of variability for populations with given fecundity and other parameters.

To consider populations that are intermediate between Crow and Morton's (1955) reference values, suppose each family is formed by a unique male and female pairing, and each family has a unique underlying probability distribution for family size. Let λ_i denote the underlying mean of family size for the ith family, and assume that conditioned on λ_i the family size is generated by a Poisson distribution. Then for the ith family, the actual family size, k_i, is a random variable with mean and variance λ_i. Let λ_i follow some distribution with probability density function $g(\lambda)$, and note that k_i, the actual family size for the ith family, is then generated by a compound distribution (Titterington et al. 1985).

We are not assuming that the actual family size follows any particular distribution. If the variance of λ is zero, then the observed family size, k, follows a Poisson distribution, and differences in family size are due to purely random processes not indexed by individual family. Alternatively, if the variance of λ is large, then the distribution of the actual family size is indexed by family. In this case, the distribution of actual family sizes has a greater variance than the Poisson distribution with the same mean, but we do not know to what extent this variation is due to genetic causes. This familial variation could be the result of genetic or nongenetic factors. For example, some redds within a stream could be destroyed by ice or other environmental catastrophe, and this will increase the nongenetic familial variation. If salmon could be raised in such a way to nearly eliminate nongenetic sources of familial variation, then variation in λ largely would be due to genetic variation. Either way, to the extent that the variation in λ increases, the variance of family size increases. When this happens, the effective population size (Wright 1931; Crow and Kimura 1970) will decrease relative to the population census, as will the amount of genetic variation the population can carry.

Using $E(\ )$ to denote mathematical expectation, the unconditional mean of k_i must equal the mean of λ_i, since

$$E(k_i) = E(E(k_i|\lambda_i)) = E(\lambda_i)$$

To find an expression for the variance of k_i, in terms of the variance of λ_i, note that $V(k_i|\lambda_i)$ is the Poisson variance. Then, using the well-known result (e.g., Lindgren 1993)

$$V(x) = V(E(x|y)) + E(V(x|y))$$

we can find $V(k)$ as

$$(1) \quad V(k) = V(E(k|\lambda)) + E(V(k|\lambda)) = V(\lambda) + E(\lambda)$$

Recalling that λ_i is the expected underlying number of offspring from the ith family, let r_i denote the expected number of offspring that survive to some life stage, such as entry into the marine environment. Now, let s_i be the *underlying survival* for this family in the next life stage, so that

$$\lambda_i = s_i r_i$$

We assume that s_i and r_i are independent, so that for C_s and C_r denoting the respective coefficients of variation in the Appendix, we show that

$$\frac{V(k)}{E(k)} = 1 + E(k)[C_r^2 + C_s^2 + (C_r C_s)^2]$$

Then, the variance-mean ratio (or for $\bar{k} \approx E(k)$ and $v(k) \approx V(k)$, the index of variability) might be inflated by (1) family-specific influences that cause the number of recruits to the marine life stage to vary or by (2) family-specific influences on survival in the marine life stage. In the absence of family-specific influences, this ratio is 1.

Methods

Identifying families of anadromous salmon

In September 1982, 22 female pink salmon (dams) were crossed with 33 males (sires) in a factorial design (Fig. 1) with 11 replicated crosses of two females with three males. Dams and sires that were judged to be fully mature were chosen and mated at random on each day that spawning occurred. All of the offspring of one female failed

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Fig. 1. Diagrammatic representation of the mating design for the early 1982 run. Sires are shown as boxes and dams as circles. Each sire was to be mated with two dams, and each dam was mated with three sires. This created six half-sibling groups per replicate.

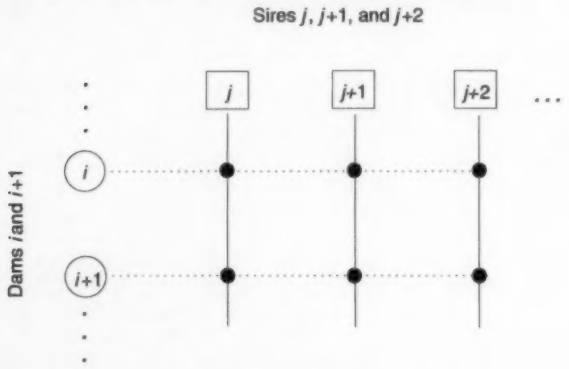
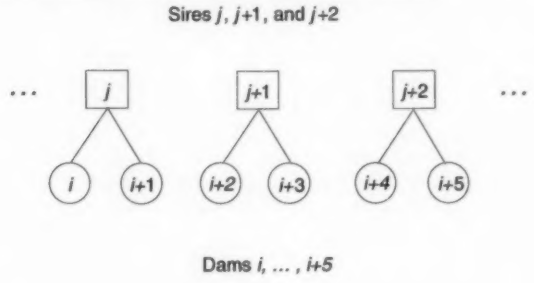


Fig. 2. Diagrammatic representation of the mating design for the 1983 and 1984 early and late runs. Sires are shown as boxes and dams as circles. Each sire was to be mated with two dams, creating a nested design, with two half-siblings groups per sire.



to survive incubation, probably because that female was not fully mature on the day she was spawned; this failed family was not included in subsequent analyses. In 1983 and 1984, similar crosses were performed, except that in these years, two dams were nested within each sire (Fig. 2) and two temporally spaced runs (Smoker et al. 1994, in press[2]) were sampled each year. We considered this arrangement as five distinct groups of salmon for this study: (1) 1982 late, (2) 1983 early, (3) 1983 late, (4) 1984 early, and (5) 1984 late. In the 1983 early and late groups, 60 females were crossed with 30 males; in the 1984 early group, 66 females were crossed with 33 males; and in the 1984 late group, 54 females were crossed with 27 males. In each group, the offspring from at least one, but no more than three, dam–sire crosses failed to thrive for reasons having to do with artificial culture; these failed offspring were not included in subsequent analyses. The total eggs for each individual female were divided into two approximately equal groups; each group was then fertilized and assigned at random to a cell in an incubator. After emergence and tagging with a group-specific half-length coded-wire tag (Thrower and Smoker 1984), all groups were placed into a common holding tank until release. The juveniles were counted and released into the ocean prior to their first exogenous feeding, at about 0.25 g wet body weight. Surviving adult fish were intercepted and counted at a trap 16 months later as they entered freshwater. All pink salmon were native to Auke Creek, Alaska (Taylor 1980; Gharrett and Smoker 1993). The mating experiments, incubation, release, and recovery occurred at Auke Creek, in Auke Creek water.

Individual pink salmon families were monitored for survival to return based on the recovery of coded-wire tags. A total of 300 individual pink salmon families were tagged as larvae (unfed fry) in the five groups. We considered marked individuals a random sample from the total offspring surviving to that time, and C_r, the coefficient of variation of recruitment to the marine life stage, was held to a minimum by striving to tag and release 1000 juveniles per family.

Computation

As a variance stabilizing measure, release and return numbers were converted to the empirical logit (Agresti 1990) for each dam–sire cross. The empirical logit then became the trait of interest. Analysis of variance was used to estimate variance components. The usual F-test for a sire effect was used to detect an additive genetic component to the variation in the trait (Becker 1984). Because of the relatively small samples sizes, and the lack of information on the size of the effect we might encounter, all tests were performed at the 0.10 level of significance (Tacha et al. 1982).

The size of a particular dam's family was not independent of the size of the family with the same sire but different dam (see Figs. 1 and 2), complicating the computation of variance of the number of offspring per family. In other words, the offspring numbers in half-sib family groups were correlated, which could result in an underestimate of variance in family size. For the 1982 families, the mean and variance of the family size were considered to be functions of the dam, and offspring numbers were summed over sire only after we failed to find a statistically significant sire effect ($P > 0.95$). In the 1983 and 1984 families, dams were randomly divided into two groups so that no sire was crossed with more than one dam in each group. We then calculated the mean and variance for each group and then averaged the mean and variance over the groups.

Results and discussion

The index of variability increased as family size increased; similarly, the significance probability associated with a sire effect generally declined (indicating less probability that the sire effect is due to random chance) as family size increased (Table 1). The sire effect in the analysis of variance table measures the genetic influences on the variability of the trait of interest (e.g., Becker 1984; Falconer 1989). Two groups had statistically significant sire effects (Table 2). The logit of marine survival, and by implication C_s, can have a nonzero genetic component in pink salmon — survival and family size are traits that can be inherited.

Family size for the five release groups appeared more dispersed than the Poisson distribution would provide, although family size did not seem to consistently follow any obvious distribution (Fig. 3). The Poisson distribution has a single parameter for both the mean and variance; data with a variance far in excess of the mean are sometimes described as overdispersed relative to the Poisson distribution. The two release groups with the highest survival, i.e., 1983 early and 1983 late, had the greatest overdispersion and the largest average family sizes.

These results provide several refinements to our understanding of pink salmon biology. Specifically, these results provide (1) insight into the effective population size (Wright 1931), a familiar genetic parameter describing the amount of

[2] Smoker, W.W., Gharrett, A.J., and Stekoll, M.S. Genetic variation in seasonal timing of anadromous migration in a population of pink salmon (*Oncorhynchus gorbuscha*). Presented at the International Symposium on Biological Interactions of Enhanced and Wild Salmonids, Nanamio, B.C., June 1991.

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Table 1. Four statistics of interest on survival within pink salmon families released into the Pacific Ocean in five distinct groups.

Statistic	1982 early	1983 early	1983 late	1984 early	1984 late
Sire significance probability	0.97	0.07	0.008	0.85	0.71
Mean survival	0.003	0.012	0.019	0.001	0.002
Mean family size	2.8	9.3	13.2	0.7	2.0
Index of variability	1.77	4.97	4.03	2.45	1.58

Note: As a variance stabilizing measure, release and return numbers were converted to the empirical logit for each dam-sire cross; the empirical logit of survival then became the trait of interest. Sire significance probably is the result of an analysis of variance test of no sire effect. Mean survival represents survival from emigration into the marine water until adult return. Mean family size is the total number of adult breeders returning divided by the number of females in the parent year. The index of variability is the variance of family size divided by the mean family size and plays an important role in determining the genetic quantity called the effective population size.

Table 2. Analysis of variance tables for the logit of marine survival of pink salmon returning to Auke Creek, Alaska.

Source	df	Sum of squares	Mean squares	Significance probability
1982 late				
Total	58	39.2439		
Sire	32	14.8873	0.4652	0.970
Error	26	24.3566	0.9368	
1983 early				
Total	58	41.083		
Sire	29	26.1007	0.9000	0.070
Error	29	14.9824	0.5166	
1983 late				
Total	56	10.7643		
Sire	29	7.9117	0.2728	0.008
Error	27	2.8526	0.1056	
1984 early				
Total	58	50.2969		
Sire	29	20.3541	0.7019	0.848
Error	29	29.9428	1.0325	
1984 late				
Total	58	42.9130		
Sire	29	19.2798	0.6648	0.706
Error	29	23.6332	0.8149	

Note: Families were individually tagged with coded-wire tags for one run in 1982 and each of two runs, an early and a late run, in 1983 and 1984. In 1982, sire and dams were crossed in a factorial design. In 1983 and 1984, families had a single dam, but sires were mated with two dams, creating a nested design. Not all crosses had offspring surviving to release.

genetic variability that a population can retain, (2) evidence for a genetic component to marine survival in some environments, and (3) the observation that in the groups of salmon we studied, the index of variability increased with the mean family size.

We implicitly assumed that the salmon were not affected by residual nongenetic, family-specific influences on survival in the marine environment. Siblings that were incubated together could conceivably remain together in the marine environment and be subjected to common mortality, or prerelease handling could have had a large family-specific postrelease effect on survival. We assume that these possibilities are remote, but in any case, they would not have produced the results we observed. The nested design used in the breeding experiment would have prevented most nongenetic influences from affecting the statistical hypothesis tests of genetic influence on survival. The statistical hypothesis tests were based on the sire effects. That is, the F-statistic in question was inflated by a consistent commonality among the survival of half-siblings with the same sire, but not the same dam, same incubator, and so forth.

For populations that are expanding or contracting, Kimura and Ohta (1971) gave the following formula for the variance effective population size:

$$N_e = \frac{(N_{t-1} - 1)\bar{k}}{R_k + 1}$$

In populations that are neither expanding nor contracting, the average family size, $\bar{k}$, must be equal to 2. Assuming no familial mortality and random mating, the variance in the family size must also be approximately 2 (Crow and Kimura 1970), so that $R_k \approx 1$ and $N_e \approx N_{t-1} - 1$. However, we observed indices of variability approximately twice as large as this model predicts, even when variation in recruitment to the marine life stage was artificially held to a minimum. In wild populations, C_r is probably very high if for no other reason than the

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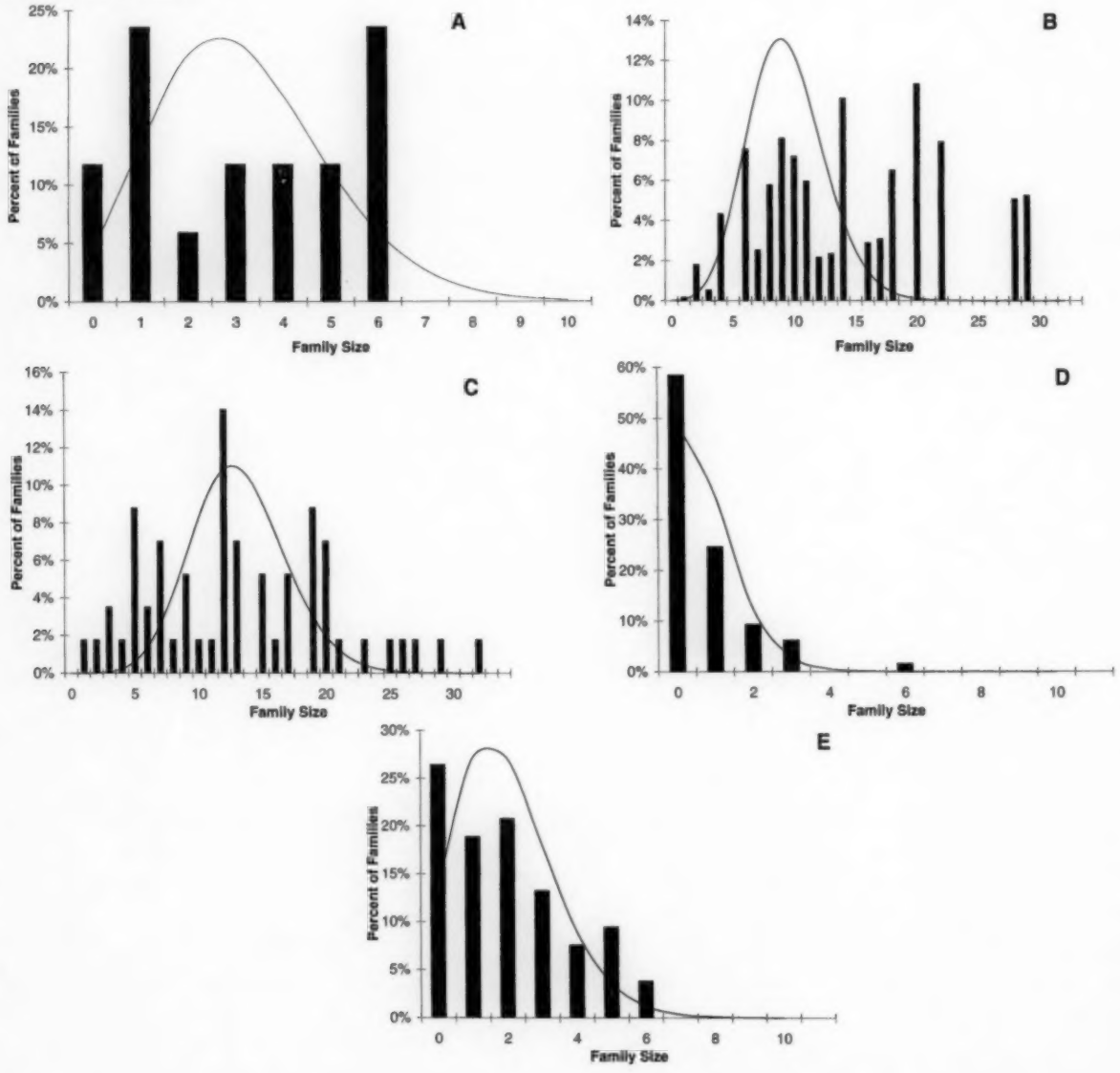
Fig. 3. Individual family size (number of returning offspring) for each female for each of five runs of pink salmon returning to Auke Creek, Alaska, for (A) the 1982 late run, (B) the 1983 early run, (C) the 1983 late run, (D) the 1984 early run, and (E) the 1984 late run. The vertical bars show the actual distribution of offspring for each female. The curves show the shape of a Poisson distribution with the same mean as the observed average family size.



physical proximity that eggs from a single family have in the redd. With the index of variability near 2, the effective population size will be near two thirds of the breeding population size. If there is a weak association between $\bar{k}$ and $v(k)/\bar{k}$, and little familial variation in recruitment to the marine life stage, hypothetical N_e/N_t ratios will slowly decline to less than 0.4 over the range of family sizes that we have observed (Fig. 4). In wild pink salmon populations, we can expect N_e/N_t to be far less than two thirds because of familial variation in recruitment to the marine environment.

Given that pink salmon may at least occasionally experience density-dependent population size regulation (McNeil 1964), our result leads to the question of why selection does not increase the productivity of the stock by favoring those fish with higher marine survival. The answer must be that the favored phenotypes change frequently between generations. Further evidence of this is supplied by McIntyre et al. (1988) who artificially selected individuals for increased marine survival in four successive broods of a coho salmon (*Oncorhynchus kisutch*) line. The authors reported that marine survival in the selected line decreased compared with a control line, presumably because of the loss of genetic variation.

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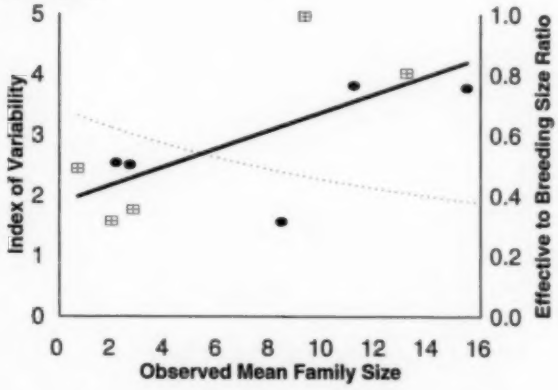
Fig. 4. Index of variability for experimentally produced salmon (left axis) as a function of mean family size. Because of similar handling in early life stages, these fish have a minimum of familial variation of recruitment to the marine lifestage. Boxes represent the five groups of Auke Creek pink salmon. Solid circles represent observations of coho salmon by Simon et al. (1986). In all cases, the index of variability exceeded 1. Functional relationships between family size and index of variability are unknown and surely differ among species, times, and locations. Because of the limited sample sizes, a single linear smoothing is provided using the estimated regression of the Auke Creek pink salmon and Simon et al. (1986) coho salmon (solid line). Based on this regression relationship, a hypothetical N_e/N_t relationship is plotted (broken line, right axis) as a function of mean family size for population sizes large enough that $N_t \approx N_t - 1$ and assuming no familial variation in recruitment to the marine life stage. Estimated regression parameters for the equation $y = \hat{a} + \hat{b}x$ are $a = 1.88$ and $b = 0.150$ for combined data (shown), $a = 2.03$ and $b = 0.102$ for the coho salmon data, and $a = 1.69$ and $b = 0.227$ for the pink salmon data.



If marine survival of salmon cannot be increased by selection, any surplus of animals available for human use comes from a changing and unpredictable fraction of the population. A genetic component of marine survival and a changing environment underscore the importance for fitness of genetic variability in exploited or recovering salmon populations.

Loss of genetic variation can reduce survival and mean fitness (Falconer 1989). Several recent studies provide examples of inbreeding, and its associated loss of genetic variation, leading to detectably reduced survival in vertebrates in natural habitats (Jimenez et al. 1994; Keller et al. 1994). Ryman (1970) reported lower recapture frequencies in inbred families of Atlantic salmon (*Salmo salar*) and indicated a likely additive genetic component of variation of recapture frequencies. In the only other study we know of in which family size was directly observed in Pacific salmon, Simon et al. (1986) tagged 158 families of hatchery-produced coho salmon over 5 years and reported the index of variability of family size. They also noted an increase in the variance of family size as a function of mean family size and observed that the family size distribution did not fit any simple distribution, such as the Poisson. Their observations closely agree with ours (Fig. 4). The index of variability was always larger than 1, and increased with average family size — a result consistent with Crow and Morton's (1955) findings with fruit flies.

Our finding that $v(k)/\bar{k}$ is larger than 1 is consistent with studies of other species. A value of $v(k)/\bar{k}$ nearly as high as 15 was reported by Begon (1977) in a laboratory study of the fruit fly (*Drosophila subobscura*). Nozawa (1972) reported effective population size estimates of 20–76% of the breeding population size in Japanese macaque (*Macaca* sp.). Ryman et al. (1981) simulated populations of moose (*Alces alces*) and whitetail deer (*Odocoileus virginianus*) and speculated that effective population sizes would be between 20 and 42% of total populations under various harvest strategies that did not alter sex ratios. Frankham (1995) recently provided a review of N_e/N in a large number of different species, including insects, mollusks, fishes and other vertebrates, and plants; this review included estimates of 0.9 for rainbow trout (*Oncorhynchus mykiss*) but 0.013–0.043 for chinook salmon (*Oncorhynchus tshawytscha*). Nunney (1995) proposed that the ratio of effective population size to breeding size "is expected to be higher than 0.5" in populations with overlapping generations, but that the lack of overlapping generations creates "an increased likelihood of extremely low values (i.e., $N_e/N <$ 0.25)."

In summary, we showed that the variation in family size of anadromous salmon is a function of two potentially important factors: (1) familial recruitment at the end of some life stage and (2) the subsequent familial survival in the marine environment. We then detected large familial differences in marine survival in the populations of pink salmon we studied and linked familial variation in family size to genetic causes. By observing the association between average family size and variance in family size, we find a link between population increase (or exploitable production, surplus to breeding needs) and genetic variability in the population. The important biological conclusion is that a relatively small part of the breeding population is the most productive and that this productive segment is both changing and unpredictable. Thus, population dynamics models that only incorporate abundance will fail to predict decreases in stock productivity as a consequence of actions that maintain abundance in the short term, but decrease genetic variability.

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Appendix

Recalling that $\lambda = sr$ and using Goodman's (1960) formula for the variance of the product of independent random variables:

$$V(\lambda) = E(s)^2V(r) + V(s)E(r)^2 + V(s)V(r)$$

Now, substituting this expression into eq. 1 and dividing by $E(k)$:

$$\frac{V(k)}{E(k)} = \frac{E(\lambda) + V(\lambda)}{E(\lambda)}$$

$$\frac{V(k)}{E(k)} = 1 + \left[\frac{E(s)^2V(r)}{E(sr)} + \frac{E(r)^2V(s)}{E(sr)} + \frac{V(r)V(s)}{E(sr)}\right]$$

$$\frac{V(k)}{E(k)} = 1 + E(sr)\left[\frac{E(s)^2V(r)}{E(s)^2E(r)^2} + \frac{E(r)^2V(s)}{E(s)^2E(r)^2} + \frac{V(r)V(s)}{E(s)^2E(r)^2}\right]$$

$$\frac{V(k)}{E(k)} = 1 + E(\lambda)[(C_r^2 + C_s^2 + (C_rC_s)^2]$$

$$\frac{V(k)}{E(k)} = 1 + E(k)[(C_r^2 + C_s^2 + (C_rC_s)^2]$$

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Zooplankton size and its relationship to trophic status in deep Ontario lakes

William D. Taylor and John C.H. Carter

Abstract: We found the size distribution of crustacean zooplankton in deep (>20 m) oligotrophic to mesotrophic lakes in southern Ontario to be highly variable. Mean lengths varied from 0.48 to 1.12 mm. This difference is not strongly correlated to community composition at the genus to family level but is related to the presence of planktivores, cisco (*Coregonus artedii*) and *Mysis relicta*. Empirical analysis of our data demonstrates that mean zooplankton length is correlated to the residuals of the chlorophyll – summer total phosphorous relationship and also to the decline in total phosphorus from spring to summer. Our analysis suggests that zooplankton size distribution plays a significant role in determining the trophic status of these lakes, and we speculate that human alteration of fish communities may affect zooplankton size distribution and trophic status.

Résumé : Nous avons observé une forte variabilité dans la distribution par taille des crustacés zooplanctoniques dans des lacs profonds (> 20 m) oligotrophes ou mésotrophes du sud de l'Ontario. Les longueurs moyennes variaient entre 0,48 et 1,12 mm. Cette différence n'est pas fortement corrélée à la composition de la communauté au niveau du genre ou de la famille, mais est en rapport avec la présence de planctonophages, le cisco de lac (*Coregonus artedii*) et la *Mysis relicta*. L'analyse empirique de nos données démontre que la longueur moyenne des zooplanctontes est corrélée aux résidus de la relation chlorophylle – phosphore total estival, ainsi qu'à la baisse du phosphore total entre le printemps et l'été. Notre analyse fait ressortir que la distribution par taille du zooplancton joue un rôle important dans le statut trophique de ces lacs, et nous pensons que la modification anthropique des communautés ichtyennes peut agir sur la distribution par taille du zooplancton et sur le statut trophique.
[Traduit par la Rédaction]

Introduction

A strong effect of vertebrate planktivores on freshwater zooplankton communities has been documented in both experimental studies, where fish communities have been altered in lakes or lake enclosures and in comparative studies, where lakes with different fish communities have been compared (Zaret 1976). Experimental studies in lakes and lake enclosures (Shapiro and Wright 1984; Mazumder et al. 1988) have demonstrated that effects of vertebrate planktivores cascade to the phytoplankton, and historical evidence from lakes has provided supporting evidence (Mills et al. 1987; Jassby et al. 1990; Horn and Horn 1995). However, results from comparative studies have been mixed, and many have found weak or no relationships between vertebrate predation (as reflected in zooplankton community composition) and phytoplankton (Mills and Schiavone 1982; Pace 1986; McQueen et al. 1986; Ramcharan et al. 1995). Exceptions generally are those studies that have sorted lakes by zooplankton size, included eutrophic lakes, and accounted for the strong effect of total phosphorus on phytoplankton biomass (Quirós 1990; Sarnelle 1992; Mazumder 1994).

One possible explanation for the apparent weakness of top-down effects on phytoplankton in comparative studies is that planktivore abundance in experimentally manipulated systems, or systems that have undergone significant shifts in their fish communities, varies more than in lakes that have not undergone recent shifts in fish community composition. Fish that consume plankton, at least for some part of their life-span, are present in most lakes. Piscivores that may depress planktivores numbers are present as well but are exploited so that real food chains in lakes are seldom clearly planktivore dominated or piscivore dominated. Therefore, variation in the intensity of vertebrate planktivory is small compared with differences in nutrient loading, and effects of vertebrate planktivory on phytoplankton are relatively constant and difficult to isolate in comparative studies.

A second possibility is that the strong effects observed in experimental manipulations of fish communities are short-term phenomena, and plankton communities adjust to minimize the effects over time (Ramcharan et al. 1995). Behavioural and population changes may occur rapidly, but relatively slow processes, such as invasion of new species and adaptation by natural selection, continue to minimize effects on lower trophic levels over time scales too slow to be observed in one-season experiments.

An often-overlooked aspect of the comparison between experimental and comparative studies is that comparative studies have considered total phosphorus (TP) to be an independent variable, and zooplankton community composition is treated as a second independent variable that might explain some of the residual variation in chlorophyll. However, experimental manipulations of fish communities may result in changes in TP (Wright and Shapiro 1984; Mazumder et al. 1989), possibly through altered sedimentation of P, transport of P by organisms, or changes in the partitioning of P among trophic levels. Sedimentation is affected by zooplankton community

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composition (Uehlinger and Bloesch 1987; Elser et al. 1995) and P decline during thermal stratification has been related to particle-size distribution (Guy et al. 1994). Further, strong effects of biomanipulation on lower trophic levels involve changes in size structure as well as biomass. Small algae are more effective at scattering light per unit mass than large algae, so that changes in water clarity can be greater than changes in chlorophyll or phytoplankton biomass.

In this study, we use a comparative approach to search for top-down effects on unmanipulated lake communities by examining zooplankton size in lakes of similar morphology (small to medium sized) and nutrient status (oligotrophic to mesotrophic). We examine first whether zooplankton mean size is associated with differences in zooplankton community composition and biomass. We then investigate whether the mean size of zooplankton is associated with the presence of certain planktivores or other factors including lake morphology, chemistry, and nutrient status. Lastly, we look for the correlation of mean zooplankton size with attributes of lakes that we might expect to be affected by top-down effects: decline in TP during stratification and chlorophyll. We conclude that zooplankton size distribution has a significant impact on total phosphorus and chlorophyll concentration in these lakes.

Materials and methods

The lakes used in this study (Table 1) are in a broad band across southern Ontario. Different basins of the same lake are treated here as separate lakes in some cases; the basins we treat as separate lakes are separated by shallow narrows and both basins are deep. On each sampling trip, we worked westward from the Kingston area to Haliburton. Lakes were sampled twice, between April 20 and 29 (immediately after ice-out) and between August 10 and 17. Sampling of these lakes began in 1986, and data reported were collected from 1986 to 1988. All were sampled at a single station at a depth of approximately 22–24 m. This was the maximum depth for some of the lakes. Some of the resulting data have been published previously (Guy et al.1994; Taylor et al. 1991), and these lakes have been used in other recent studies of zooplankton ecology (Almond et al. 1996).

Morphological features of the lakes were mostly from data accompanying bathymetric maps published by Ontario Ministry of Natural Resources (OMNR) for sport fishermen. Only preliminary maps were available for some lakes, and measurements were taken from these by planimetry.

On each spring visit to the lakes, integrated water samples (0–10 m) were collected for the determination of TP with a polyethylene tube. On each summer visit, integrated water samples were collected for determination of TP, chlorophyll *a*, and other chemical measures not reported here. Colour, conductivity, pH, and turbidity were measured in the field with portable meters. Secchi depth was estimated with a 25-cm Secchi disc. Chlorophyll and TP were done at University of Waterloo. On some trips, additional analyses of chlorophyll and TP were performed by Environment Canada, Burlington, Ont. Because of the similar trophic status of the lakes, six replicate analyses of TP and three replicate analyses of chlorophyll *a* were performed. TP was estimated after the samples were autoclaved with persulfate, using the ascorbic acid modification of the molybdenum blue method (Parsons et al. 1984). Chlorophyll samples were collected on Whatman GF/F filters, extracted in dimethylsulfoxide and acetone (Burnison 1980) and phaeopigments were estimated by change in absorbance following acidification as in Lorenzen (1967).

We found that phaeopigment and chlorophyll *a* were variable among replicates, but their sum was relatively constant. This probably reflects problems of preservation of chlorophyll during our long sampling trips, despite our having filtered the samples and put the filters on dry ice within 10 h of collection. To correct for this problem, we report the sum of the two pigments. That is, when the change in absorbance on acidification was ≥40%, we did not correct the chlorophyll estimate. However, when the change in absorbance was <40%, indicating presence of phaeopigments, we estimated their concentration with the Lorenzen (1967) formula and added this amount to the corrected chlorophyll value.

On summer trips, three replicate zooplankton hauls were collected with a 0.5 m diameter, 64 µm mesh metered zooplankton net. Samples were taken from just above the bottom to the surface in 22–24 m of water. The efficiency of each haul was measured with a flow meter mounted at one third of the distance between the rim and the centre of the net opening. Zooplankton were preserved in buffered formalin and later enumerated and measured. About 200 individuals were counted and measured from each replicate. Biomass was estimated with length–weight regressions, as employed by the ZEBRA zooplankton counting program used by Ontario Ministry of the Environment, and corrected for net efficiency.

In preparing for the multivariate analysis of zooplankton community composition, the among-lake variation in biomass of the different zooplankton taxa and total biomass was examined using a plot of the log(variance) to log(mean); the slope was 1.8, so we elected to use a logarithmic transformation on all biomass data (Taylor 1961).

Results

The mean length of crustacean zooplankton in our lakes ranged from 0.48 (Charleston Lake) to 1.12 mm (Salmon Lake). The average weight of crustacean zooplankton in these two lakes was 1.3 and 11.2 µg. The mean of lake means for zooplankton mean length was 0.74 mm. The largest animals in these samples were mostly Cladocera (Fig. 1). Mean length of zooplankton was negatively correlated with the contribution of cyclopoids ($r = -0.56$) and *Diaphanosoma* ($r = -0.53$). The relationship with *Daphnia* was positive and almost significant ($r = 0.300$, critical $r = 0.305$). Calanoids were the most abundant crustacean zooplankton and contributed most of the biomass in most lakes. Their biomass is not correlated with mean length.

We examined the value of mean length as a summary of zooplankton composition by performing a principal components analysis (PCA) on zooplankton biomass data (Table 2). Length was correlated to the first four principal components. By summing the products of the squared correlations and the eigenvalues from the PCA, we estimate that zooplankton length expresses some 13% of the variation in the log-transformed zooplankton biomass data.

Mean length of zooplankton was not significantly related to chemical or morphological features of the lakes (conductivity, pH, dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC), area, mean depth, maximum depth, or shoreline development). The highest correlation was with colour ($r = 0.27$, $P > 0.05$), although only a few of the lake basins were noticeably coloured. (Colour as Hazen units was estimated as <5 in 26 lakes, 5–15 in 15 lakes, and 30 in 1 lake.)

The mean length of crustacean zooplankton was related to the presence of invertebrate and vertebrate planktivores. Cisco (*Coregonus artedii*) was present in 32 of our lakes (OMNR, published lake summary sheets and unpublished data at district offices). The mean length of zooplankton was smaller in lakes with cisco (0.713 mm) compared with lakes without cisco (0.841 mm). This difference is significant (ANOVA, $P <$

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Table 1. Characteristics of the lakes.

Lake	Latitude (N)	Longitude (W)	Area (km^2)	Z_m* (m)	Z^* (m)	Spring TP ($\mu g \cdot L^{-1}$)	Summmer TP ($\mu g \cdot L^{-1}$)	Chlorophyll *a* ($\mu g \cdot L^{-1}$)	Mean zooplankton length (mm)
Bob's									
East basin	44°41′	76°35′	12.6	23	6	13.1	10.3	3.0	0.74
Green Bay	44°41′	76°35′	4.8	25	14	11.0	8.2	2.5	0.74
West basin	44°41′	76°35′	9.4	23	10	12.6	9.7	2.5	0.56
Boshkung	45°04′	78°44′	6.4	71	23	3.9	4.7	2.5	0.59
Brulé	45°04′	76°26′	5.1	56	22	6.9	5.3	1.3	0.73
Buck									
North basin	44°31′	76°28′	2.6	32	11	11.9	13.3	5.9	0.72
South basin	44°32′	76°26′	6.7	41	12	13.1	9.6	2.6	0.80
Buckshot	45°00′	77°04′	4.0	33	10	9.1	7.5	3.3	0.88
Chandos	44°49′	78°00′	15.6	48	13	12.6	10.2	2.8	0.73
Charleston	44°32′	76°00′	22.6	91	17	16.9	15.4	2.3	0.48
Crow	44°43′	76°37′	4.3	35	15	9.0	8.3	3.5	0.66
Desert	44°33′	76°35′	3.4	71	23	10.7	8.3	3.2	0.68
Devil	44°35′	76°27′	9.5	44	14	13.0	8.4	1.6	0.72
Drag	45°05′	78°24′	9.0	55	18	2.9	5.8	2.4	0.70
Eagle	44°41′	76°42′	5.8	35	12	14.6	11.4	2.0	0.73
Eels	44°54′	78°08′	8.5	30	7	6.6	6.4	1.2	0.90
Halls	44°07′	78°45′	4.9	80	30	3.7	4.5	1.3	0.80
Little Hawk	45°09′	78°43′	3.7	86	30	1.8	2.8	1.0	0.72
Jacks	44°44′	78°03′	5.4	44	16	14.1	10.0	2.4	0.78
Kashagawigamog	44°58′	78°35′	7.3	39	13	6.3	5.8	2.8	0.73
Kennebec	44°44′	76°58′	5.3	31	8	13.2	9.2	3.0	0.70
Knowlton	44°27′	76°37′	1.6	34	10	11.8	7.4	1.8	0.72
Koshlong	44°59′	78°29′	3.6	42	10	2.8	4.6	9.9	0.56
Lavallee	44°57′	77°56′	0.8	31	13	9.8	8.1	2.2	1.00
Limerick	44°53′	77°37′	6.7	29	8	10.3	7.7	1.6	0.71
Mississaugua	44°42′	78°19′	5.3	39	18	5.3	4.8	0.8	0.90
Mississagagon	44°52′	77°06′	3.4	26	11	9.4	7.6	1.2	0.70
Maple	45°06′	78°40′	3.0	36	12	6.5	5.7	2.1	0.93
Mazinaw									
North basin	44°45′	77°1.2′	11.6	145	34	3.8	4.4	1.5	0.68
South basin	44°53′	77°11′	5.0	81	12	5.7	5.0	1.4	0.74
Mountain	44°59′	78°43′	2.9	31	13	4.4	4.5	2.6	0.68
Paudash (north)	44°59′	78°04′	3.0	46	23	4.4	6.0	1.7	0.75
Redstone	44°11′	78°32′	10.7	82	22	4.3	5.4	1.1	0.87
Salmon	44°49′	78°27′	1.5	30	11	15.6	6.3	1.0	1.12
Sharbot									
East basin	44°46′	76°41′	7.4	31	5	13.9	9.2	2.4	0.81
West basin	44°46′	76°41′	6.4	32	8	10.0	11.2	4.8	0.71
Shebomeka	44°54′	77°08′	2.5	30	16	10.9	7.5	3.7	0.63
Silver	44°50′	76°36′	2.2	24	10	14.1	13.7	3.4	0.57
Sydenham	44°25′	76°33′	4.1	36	10	12.8	16.3	4.5	0.65
Weslemkoon	45°02′	77°25′	17.6	55	9	5.5	6.1	1.4	0.85
Wolfe	44°41′	76°30′	8.6	31	10	19.4	11.3	2.9	0.79
Wollaston	44°51′	77°51′	3.3	32	9	10.1	8.0	2.4	0.79

*Z_m, Maximum depth; Z, mean depth.

0.002) and accounts for about 21% of the variation in zooplankton mean length.

We obtained presence–absence data on *Mysis relicta* from Dadswell (1974) and from Paterson (1996). *Mysis* was present in 21 lakes, and the mean length of zooplankton in lakes with *Mysis* was 0.698 mm, less than lakes without *Mysis* (0.793). This difference is significant (ANOVA, $P = 0.009$) and accounts for about 14% of the variation in mean zooplankton length. *Mysis* was only found in lakes with cisco, so the effects are not independent. In a two-factor ANOVA, their combined effects accounted for 29% of the variation in mean length.

Our vertical hauls provided abundance estimates for *Chaoborus* in the water column. The correlation between mean length of zooplankton and $\log(n + 1)$ of *Chaoborus* abundance was positive and significant ($r = 0.53$). Lakes with *Chaoborus* in the water column during the day tended to have

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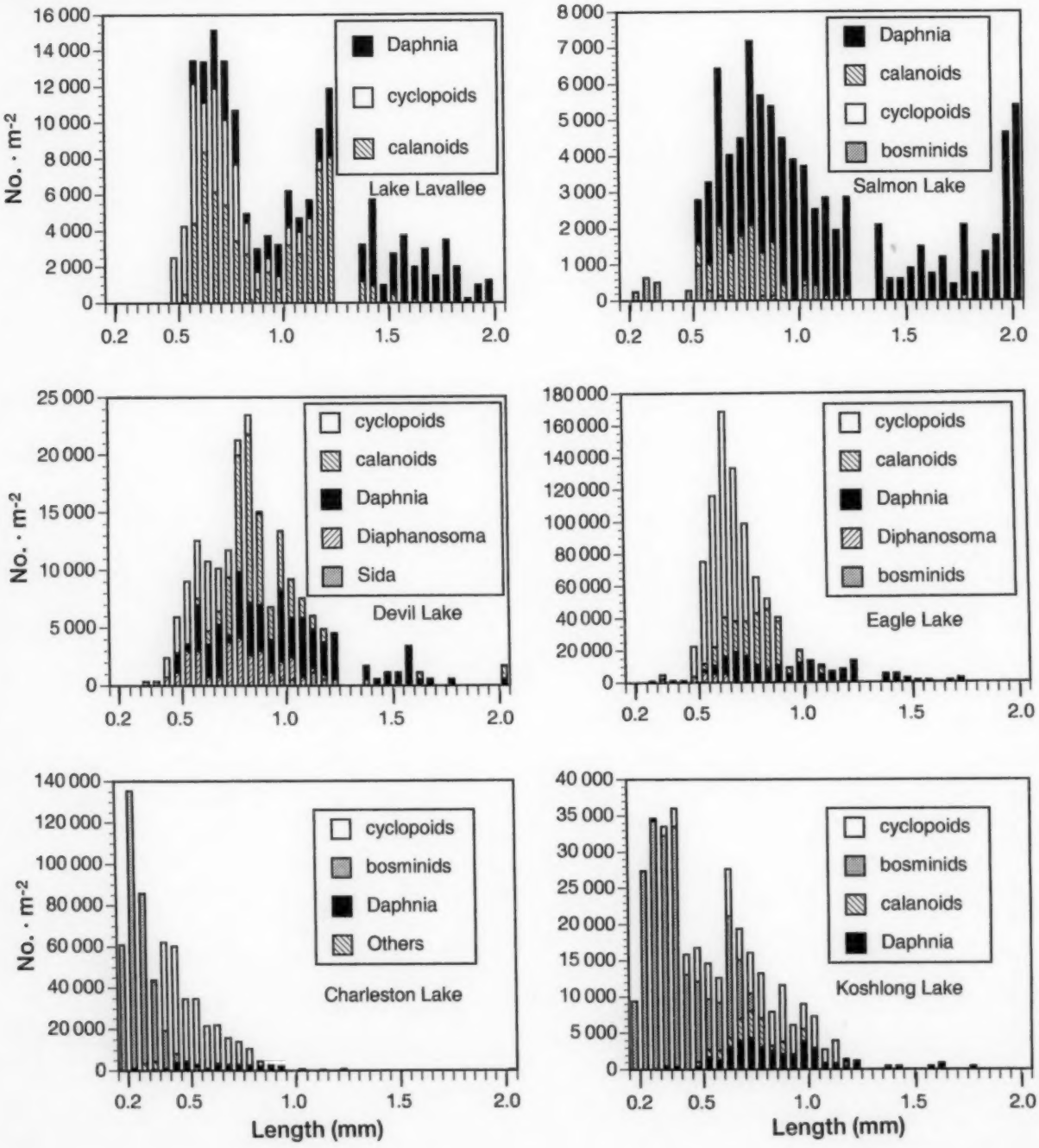
Fig. 1. Zooplankton length distributions for the two lakes with the largest mean zooplankton length (Lavallee and Salmon), two lakes at the median (Devil and Eagle), and two lakes with the smallest mean zooplankton length (Charleston and Koshlong).



larger zooplankton (mean 0.780 mm) than those without (mean 0.713 mm), but this difference was not significant (ANOVA, $P = 0.07$).

Lastly, we examined the relationship between zooplankton mean length and variables expected to indicate top-down effects on phytoplankton. Mean length was negatively related to chlorophyll ($r = -0.42$) and chlorophyll/TP ratio ($r = -0.31$), as one would expect if large zooplankton reduced phytoplankton

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biomass. There was no relationship between zooplankton mean length and water clarity, measured either as Secchi depth or as turbidity. Zooplankton mean length was not correlated with spring TP, but was correlated with the decline in TP from spring to late summer ($r = 0.31$) and with summer TP ($r = -0.31$).

To quantify these effects further, we used regression analyses to examine the effect of TP and zooplankton length on chlorophyll and on decline in TP between spring and summer. In examining these effects on chlorophyll, we found it useful to log transform both chlorophyll and TP, as has been done in other empirical studies, to increase the coefficient of determination and reduce the number of lakes identified as outliers.

Spring TP is a good predictor of summer TP (Fig. 2, upper left). With one outlier removed from the data set (Sydenham Lake, a lake with small zooplankton whose TP increased from spring to summer) the adjusted coefficient of determination is 0.77. The regression equation is

$$\ln(\text{summer TP}) = 0.731 + 0.602 \ln(\text{spring TP})$$

As zooplankton mean length was correlated to summer TP also, and to the difference between spring and summer TP, we examined its relationship to the residual variation in summer TP from spring TP (Fig. 2, middle left). That was also significant, ($r = -0.52$, $P < 0.05$) so we constructed a multiple regression. That regression was

$$\ln(\text{summer TP}) = 1.308 + 0.575 \ln(\text{spring TP}) - 0.709(\text{length})$$

Both independent variables made significant contributions ($P < 0.001$, $r^2 = 0.82$), although comparison of the standardized regression coefficients indicates that spring TP (0.89) makes a much stronger contribution than zooplankton length (–0.23). Predictions of this regression for three values of mean length are illustrated at the bottom left of Fig. 2.

Summer chlorophyll was strongly related to summer TP (Fig 2, upper right). The coefficient of determination was 0.42, with one outlier removed (Koshlong Lake). Because zooplankton length was correlated also with chlorophyll, we examined its relationship with the residuals (Fig. 2, middle right). That correlation was significant ($r = -0.32$) so we built a multiple regression model:

$$\ln(\text{chlorophyll}) = 0.100 + 0.700 \ln(\text{summer TP}) - 1.00(\text{length})$$

Both summer TP ($P = 0.001$) and length ($P = 0.04$) contributed significantly, and the coefficient of determination was 0.47. The standardized regression coefficients were 0.59 for summer TP and –0.26 for length.

From the above analyses, we were encouraged to predict summer chlorophyll from spring TP and zooplankton mean length:

$$\ln(\text{chlorophyll}) = 1.163 + 0.361 \ln(\text{spring TP}) - 1.561(\text{length})$$

The result was a model very similar to what is obtained by algebraically combining the two multiple regressions above. Both independent variables made a significant contribution ($P < 0.003$), and the standardized regression coefficients are –0.41 (for length) and 0.43 (for spring TP). The coefficient of determination ($r^2 = 0.34$) is similar to the product of the two coefficients from the summer TP and the chlorophyll models provided above ($0.82 \times 0.47 = 0.39$). The predictions of this combined model (Fig. 3) illustrate the net effect of zooplankton mean length on chlorophyll.

Table 2. Four principal components of variation in zooplankton biomass composition and their correlation with mean zooplankton length.

Taxon	1	2	3	4
Calanoids	0.230	–0.056	0.105	0.302
Cyclopoids	0.317	0.300	–0.175	0.061
Daphnia	–0.053	–0.065	0.028	0.199
Diaphanosoma	0.811	0.169	0.072	–0.088
Bosmina	–0.184	0.525	–0.436	0.044
Holopedium	–0.232	0.435	0.370	–0.182
Chydorus	0.169	–0.044	–0.046	–0.117
Leptodora	0.070	0.013	0.030	–0.130
Ceriodaphnia	0.017	0.068	0.070	–0.010
Sida	–0.053	0.364	0.299	0.219
Eigenvalue (%)	30.5	23.7	15.3	8.3
Correlation	–0.505	–0.299	0.365	0.337

Note: The principal components are unrotated and were extracted from a covariance matrix.

Discussion

The range in zooplankton mean length in our lakes was about 2-fold and corresponds to a 10-fold range in mean individual weight. Figure 1 indicates that there are major differences among these lakes in the abundance of zooplankton of a size that can be used profitably by visually feeding fish (about 1 mm). Although *Daphnia*-dominated lakes had the largest mean zooplankton size, calanoids were the most important group overall, and their abundance was not related to zooplankton size. Hessen et al.(1995) found that calanoid (and cladoceran) biomass was not strongly correlated to fish community structure. Calanoids are less susceptible to capture by piscivores such as cisco (Link 1996). Our correlation analyses and PCA lead us conclude that mean length of crustacean zooplankton is not completely independent of the relative abundance of zooplankton taxa, but neither is it primarily a reflection of community composition at the genus and family level. Similarly, Almond et al. (1996) concluded that the presence of certain planktivores affected zooplankton size, but had little discernible impact on community composition at the genus level.

Size selective planktivory has been implicated as a major force in determining the size structure of zooplankton communities (Brooks and Dodson 1965; Zaret 1976; Ramcharan et al. 1995). Our data indicate that the presence of cisco and *Mysis* is associated with smaller zooplankton. There may be greater impacts of planktivory than our data suggest, because we have not accounted for all vertebrate planktivores. For example, Charleston Lake has the smallest zooplankton and is the only lake in the data set with alewife (*Alosa pseudoharengus*). All of these lakes have yellow perch (*Perca flavescens*) and other species that have planktivorous life stages. Thus presence–absence of *Mysis* and cisco may be only weakly related to the intensity of vertebrate planktivory.

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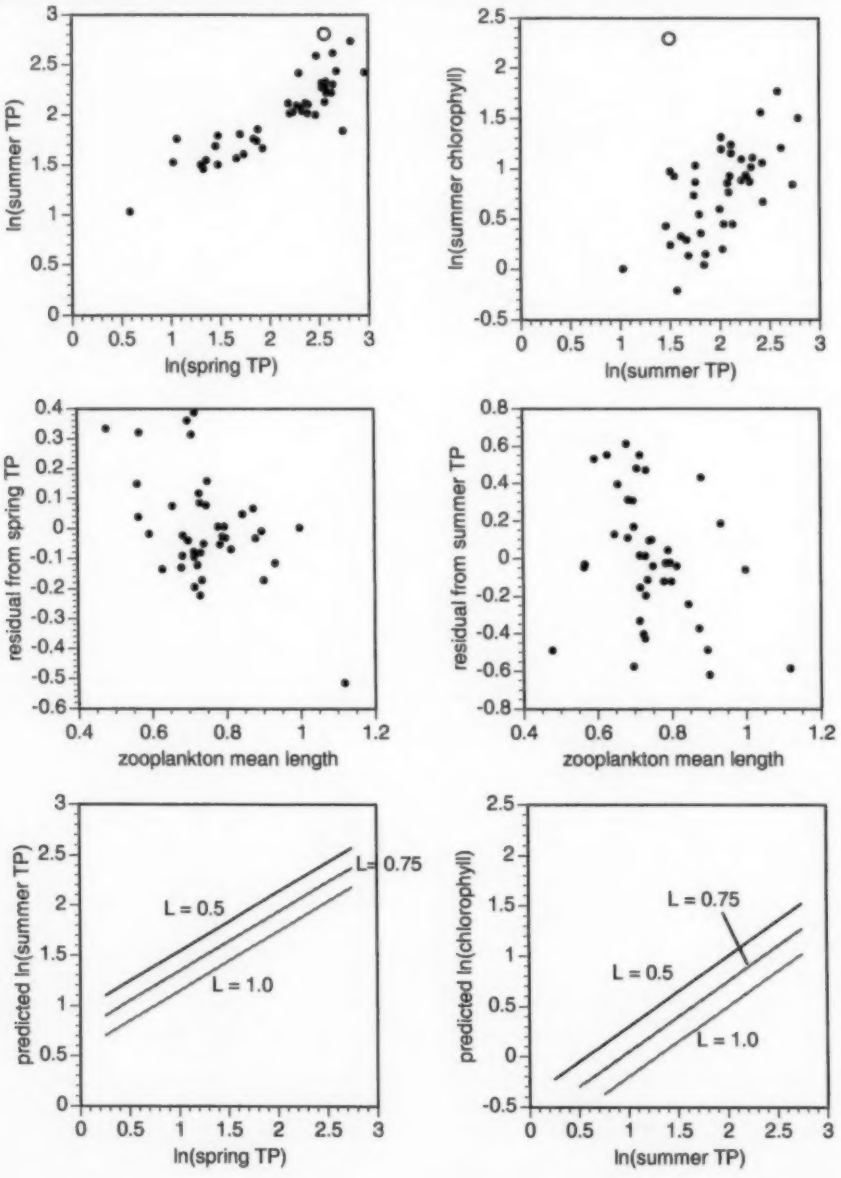
Fig. 2. Regressions illustrating the relationship of summer TP with spring TP and zooplankton length (left panels) and summer chlorophyll concentration with summer TP and zooplankton length (right panels). The units for TP and chlorophyll are $\mu g \cdot L^{-1}$ (transformed as natural logarithms), and zooplankton length is in millimetres. Data identified as outliers and not used in the regressions are shown with open circles.



Chaoborus, unlike cisco and *Mysis*, are positively correlated with the mean length of crustacean zooplankton. This is consistent with *Chaoborus* being a tactile rather than a visual predator (Pastorok 1980), preying upon smaller prey, and being preyed upon by cisco.

This study confirms that the effect of size-selective planktivores extends to the phytoplankton trophic level; zooplankton mean length was negatively correlated with chlorophyll and the chlorophyll/TP ratio, and as a second independent variable, it improves the chlorophyll versus summer TP regression. The scatter in these data is large, possibly because we measured summer TP, chlorophyll, and zooplankton length only on a single occasion. Zooplankton mean length (Mills and Schiavone 1982) and overall particle-size distribution (Guy et al. 1994) show relatively little temporal variation within lakes. However, although variation within the summer season in TP and chlorophyll is smaller than variation among lakes, several determinations are recommended to reduce error from short-term temporal heterogeneity (Hanna and Peters 1991).

That the measurement of TP is not without error has

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important implications for these analyses (Prairie et al. 1995). First, that summer TP is measured with error means that the regression coefficients of the spring TP – summer TP relationship and the summer TP – chlorophyll relationship may be underestimated. We have no independent measure of the error variance associated with summer TP. The measurement error as indicated by our replicates is relatively small, but the magnitude of the real error variance introduced by spatial and temporal heterogeneity is considerable, as can be seen by examining the trajectories for TP in Guy et al. (1994) produced by similar sampling and analytical methods.

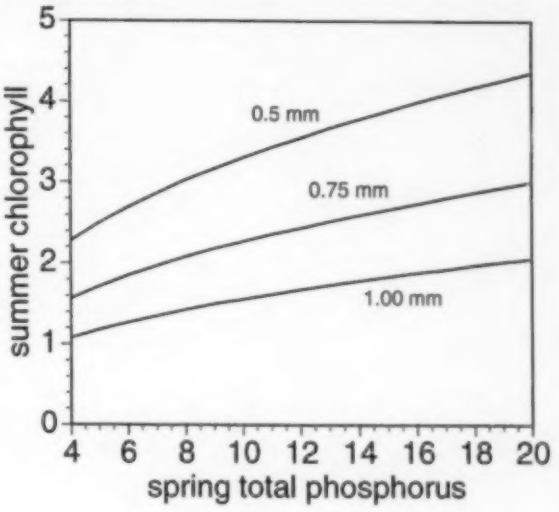
A second and potentially more serious problem arises from the possible underestimation of these regression coefficients. A second independent variable that is colinear with summer TP could appear significant only because the real effect of summer TP is underestimated. Although the relationship between zooplankton mean length and the logarithm of summer chlorophyll is not significant ($r = -0.23$), the weak relationship between length and the residuals of chlorophyll from summer TP (Fig. 2, middle right panel) could be an artifact. We do not believe this to be the case for two reasons. This relationship is expected on functional grounds and is supported by experimental and other empirical studies. Second, the importance of length persists when spring TP is used as the other predictor, and that variable is not correlated with zooplankton length (–0.018).

Scatter in the data aside, the magnitude of the effect of zooplankton size on summer chlorophyll appears to be only modest, as the regression coefficient estimates that the effect associated with an increase in zooplankton mean length from 0.5 to 1.0 mm, almost the entire range in our data set, would be a 60% increase in chlorophyll. However, our analysis also indicates that the total impact of zooplankton on chlorophyll, including its effect on chlorophyll directly plus indirectly via changes in TP, is potentially much larger.

Manipulating fish communities to reduce planktivory may lead to decreased TP, probably through increased sedimentation and partitioning of P into larger organisms (fish, large zooplankton) under-represented in TP samples. Decline in TP through the stratified season in a set of lakes overlapping the present data set was found to be correlated with plankton size distribution (Guy et al. 1994). We suggest that the pattern revealed by our data (no correlation between spring TP and zooplankton length, a positive correlation between decline in TP and length, and a negative correlation between summer TP and length) are all consistent with enhanced loss of TP during stratification in lakes with low planktivory and large zooplankton.

Another way of looking at these relations is that the strength of the summer TP versus chlorophyll relationship overestimates the importance of bottom-up effects, because summer TP is influenced by top-down forces (Persson et al. 1992; Mazumder and Lean 1994; Elser et al. 1995). Spring TP versus summer chlorophyll, a weaker relationship than summer TP versus summer chlorophyll in these data and others, may be a better indication of the strength of bottom-up forces, because spring TP is determined more by nutrient loading and influenced less by biological processes in the water column. However, it is possible that it too may be influenced by food-web effects. Sedimentation and decline in TP during stratification is generally greater than retention of P by the sediments (Dillon et al. 1990), but retention might also be affected by direct or indirect means with changes in visual predators (Brabrand et al. 1990). The importance of nutrient supply to phytoplankton biomass, the bottom-up effect, is undoubtedly important based on case studies where nutrient loading has been altered but difficult to assess in comparative studies that use surrogate variables such as TP for the external loading.

Fig. 3. Predictions of summer chlorophyll from spring total phosphorus for three different values of zooplankton mean length. Both axes are $\mu g \cdot L^{-1}$.



In other studies of top-down effects, Secchi depth has shown a stronger response to zooplankton grazing than chlorophyll. Nanoplankton are most affected by grazing, and net plankton may actually be favoured, while light scattering is predominantly by nanoplankton (Mazumder and Taylor 1994). We found that Secchi depth was not correlated with zooplankton length. This is probably because colour has an important effect on light penetration in these lakes (A. Pérez-Fuentejaja, P.J. Dillon, N.D. Yan, and D.J. McQueen, unpublished data) and offers protection from visual planktivores. The net effect is that coloured lakes tend to have large zooplankton but shallow Secchi depth.

Although our data provide support for the hypothesis that differences in zooplankton size distribution, probably attributable to differences in the intensity of visual planktivory, account for some of the variability in chlorophyll standing stock in these lakes, most of the lakes we sampled had similar values of zooplankton mean length. The mean of lake means was 0.74 mm, and the SD was 0.12. Therefore, the extremes of 0.5 mm and 1.0 mm we used for illustration are within the range we observed, but about 2 SDs away from the mean. We do not know what the zooplankton size distribution was in these lakes before human alteration of the fish communities.

Some of the variation in zooplankton mean length in these lakes is associated with presence and absence of certain effective planktivores, but how much variation is associated with quantitative differences in the abundance of planktivores because of top predators is unknown. Fish populations are exploited by sport fisheries in all of the lakes, and many have lost

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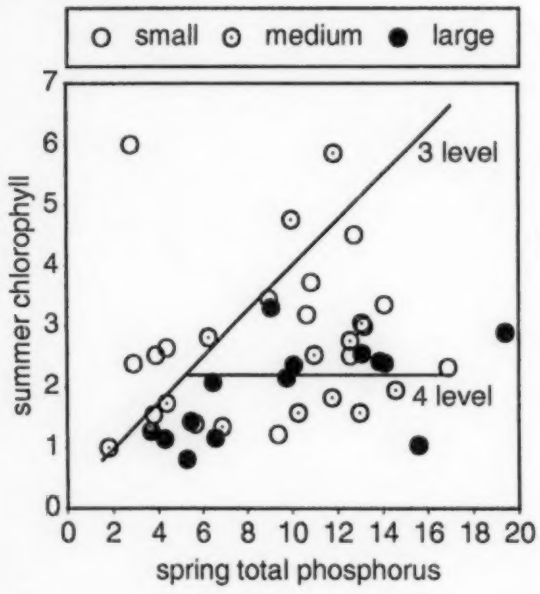
Fig. 4. The relationship of spring TP to chlorophyll in summer for lakes with large (>0.75 mm), medium (0.7–0.75 mm), and small (<0.7 mm) zooplankton. Lines show the expected form of the relationship of potential productivity and producer biomass for three and four trophic level food webs.



their lake trout (*Salvelinus namaycush*), the native deep-water piscivore. Lake trout is capable of regulating cisco and other planktivores, so this control has been relaxed to some degree in these lakes. Trippel and Beamish (1993) compared the trophic structure of several lakes with lake trout and cisco in northern Ontario. One of their lakes, Greenwich Lake, had abundant lake trout, few cisco, moderate densities of *Mysis*, and abundant large *Daphnia*, indicating that lake trout can regulate these planktivores to allow large zooplankton to exist. In more productive lakes with percid and centrarchid planktivores, Mills and Schiavone (1982) demonstrated that planktivore abundance and the size structure of fish communities was related to zooplankton size distribution and advocated the use of zooplankton mean length as a tool for fisheries managers to assess predator–prey balance in lakes.

Another way of looking at the interacting effects of nutrients and food-web effects is through simple food-web models. These models predict that three trophic level systems (i.e., phytoplankton, herbivores, planktivores) should demonstrate a positive relationship between nutrient supply and phytoplankton. Lakes with pelagic piscivores, a fourth trophic level, should not demonstrate a positive correlation between nutrient supply and phytoplankton biomass, as increasing nutrients translate into increased biomass of zooplankton and piscivores (Persson et al. 1992). Although lake trout and other predators are present in the lakes we studied, it is possible that exploitation reduces piscivory and makes them functionally more like three trophic level systems. Variation in zooplankton length should reflect the degree to which a pelagic system is acting as a three- or a four-level system. Assuming that chlorophyll is a surrogate for productivity and that spring TP is a surrogate for nutrient supply, our data lend some support to these predictions (Fig. 4) and suggest that most of the lakes are behaving as four trophic level systems.

We conclude that changes in the size structure of zooplankton populations could produce changes in trophic status for lakes in this southern region of the Canadian Shield. The most likely changes, smaller size of zooplankton because of introduction of planktivores and exploitation of piscivores, would lead to increases in summer chlorophyll and TP. However, paleolimnological assessments suggest that TP has not increased in most lakes in the study area, and it has declined in many of the richer lakes (Hall and Smol 1996). We concur with Mills and Schiavone (1982) that zooplankton size information may be a simple and useful tool for monitoring lake communities.

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Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: a test and evidence of temporal stability

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Abstract: The genetic difference between inshore overwintering Atlantic cod (*Gadus morhua*) from Trinity Bay, Newfoundland, and offshore overwintering cod from the Grand Bank region (Ruzzante et al. 1996; Can. J. Fish. Aquat. Sci. **53**: 634–645) has remained stable during 1992–1995. Cod collected inshore during 1995 in Trinity Bay ($N = 150$) were again genetically distinguishable (using $(\delta\mu)^2$ and D_{SW}) from offshore cod ($N = 140$) collected between 1992 and 1994 and were genetically indistinguishable from inshore cod ($N = 123$) also collected between 1992 and 1994. Farm-held cod ($N = 30$), captured inshore in 1992 and pen reared until 1995 were most likely to have been drawn from an inshore overwintering population; they were genetically different from offshore cod and were indistinguishable from wild inshore cod showing high antifreeze activity and from cod collected in relatively cold (~0.2°C) water in December 1995. The farm-held cod were genetically different from cod collected in relatively warm (~3.3°C) water but were indistinguishable from cod with low antifreeze activity. Despite evidence of weak genetic heterogeneity within the pool of wild cod collected inshore in 1995, which was not associated with antifreeze activity nor with water temperature, the magnitude of the genetic differences between inshore and offshore cod has remained unchanged during the period 1992–1995.

Résumé : Les différences génétiques entre la morue franche (*Gadus morhua*) hivernant dans les eaux côtières de la baie Trinité, à Terre-Neuve, et celles hivernant dans les eaux du large dans la région du Grand Banc (Ruzzante et al. 1996; J. Can. Sci. Halieut. Aquat. **53**: 634–645) sont demeurées stables entre 1992 et 1995. Les morues capturées dans les eaux côtières en 1995 dans la baie Trinité ($N = 150$) se distinguaient encore sur le plan génétique (à l'aide de $(\delta\mu^2)$ et de D_{SW}) des morues du large ($N = 140$) capturées entre 1992 et 1994, et ne se distinguaient pas génétiquement des morues des eaux côtières ($N = 123$) également prélevées entre 1992 et 1994. Des morues maintenues en captivité ($N = 30$), capturées dans les eaux côtières en 1992 et élevées dans des parcs de filets jusqu'en 1995, provenaient très vraisemblablement d'une population qui hiverne dans les eaux côtières; elles étaient génétiquement différentes des morues du large, et ne se distinguaient pas des morues sauvages des eaux côtières présentant une forte activité antigel, et des morues capturées dans une eau relativement froide (environ 0,2° C) en décembre 1995. Les morues gardées en captivité étaient génétiquement différentes d'autres morues capturées dans une eau relativement chaude (environ 3,3° C), mais ne se différenciaient pas des morues présentant une faible activité antigel. Malgré des indications d'une faible hétérogénéité génétique sur l'ensemble des morues sauvages capturées dans les eaux côtières en 1995, qui n'était pas associée avec l'activité antigel ni avec la température de l'eau, l'ampleur des différences génétiques entre les morues des eaux côtières et du large est demeurée identique pendant la période 1992–1995.
[Traduit par la Rédaction]

Introduction

Determining whether or not the genetic structure of a stock complex remains stable over time is of primary importance for the proper management and conservation of genetic resources in a marine fishery. This is of particular importance when dealing with local, or neighbouring, fish populations among which there may not be any readily apparent geographic or oceanographic barrier to mixing. A temporally stable genetic structure implies that existing, separate breeding components persist over time and are thus likely to experience independent population dynamics. For structured natural populations (a stock complex) under exploitation, failure to estimate parameters such as population-specific growth rates and fishing mortality rates separately for each component (e.g., Myers et al. 1997) can lead to the rapid elimination of those components most easily captured. This can result in detrimental effects, genetic and otherwise, not only to the different components, but to the stock complex as a whole.

Ruzzante et al. (1996*a*) described evidence of population structure within the northern cod (*Gadus morhua*) complex; specifically between cod overwintering inshore in Trinity Bay, Newfoundland, and those overwintering offshore in the North

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Cape region of the Grand Bank. The two subpopulations were genetically distinct despite the fact that individuals from both regions intermingle during most of summer and fall as a result of the inshore feeding migration of offshore cod. However, their study, and that of Bentzen et al. (1996) using virtually identical techniques, provided very limited evidence of temporal stability in the structures resolved within the northern cod complex. The dissemination of results in both of these studies led critics to rightly challenge us to demonstrate temporal stability in the defined structure. Thus, in this paper we address the question of temporal stability by comparing genetic information from new cod samples collected inshore in 1995 in the same general region of Trinity Bay as in Ruzzante et al. (1996*a*) with three different sets of samples: (*i*) those collected inshore during the period 1992–1994; (*ii*) those collected offshore during the same period, both of which were reported in Ruzzante et al. (1996*a*); and (*iii*) those more recently collected in 1995 from cod that had been caught in 1992 in the inshore region of Trinity Bay and had been pen reared in Gooseberry Cove since then (see Wroblewski et al. 1996). To maintain consistency with our earlier study we again employ the same nuclear DNA microsatellite loci as the genetic markers. For cod collected inshore we again used antifreeze protein activity in the blood (expressed as thermal hysteresis), time of sampling, and the available thermal habitat as indicators of overwintering grounds. We subsequently distinguish among the populations using the $(\delta\mu)^2$ and D_{SW} estimates of genetic distance (Goldstein et al. 1995; and Shriver et al. 1995, respectively), as well as R_{ST} (Slatkin 1995) and F_{ST} estimates of population structure.

Materials and methods

Tissue collections

Blood from cod was used for estimating antifreeze activity and as the primary source of nuclear DNA for genetic analysis. Blood (1 mL) was collected from live or recently dead cod using a sterile 2-mL hypodermic needle (21 gauge) inserted ventrally into the blood vessels that run in the haemal arches of the vertebrae between the anal fin and the caudal peduncle. If the sample was destined entirely for genetic analysis, it was preserved immediately in 5 mL of 95% ethanol and stored. Alternatively, approximately one half was preserved in ethanol, and the remainder, to be used for antifreeze analysis, was injected directly into Vacutainers (Becton Dickinson™) containing sodium heparin, mixed gently, and held on ice until centrifuged. Samples were centrifuged for 10 min at 4000 × *g*, the blood plasma was removed, placed in 1.5-mL Eppendorf tubes, and stored until analysed. Soft muscle tissue taken from the posterior of the tongue and preserved in alcohol was employed for DNA extraction when blood was unavailable.

Blood plasma antifreeze and thermal hysteresis

Data on antifreeze activity within each plasma sample were obtained using the protocol described in Goddard et al. (1994). Briefly, the blood plasma freezing and melting points were determined by microscopic observation of the freezing and melting behavior of a single ice crystal within the plasma. The difference is termed thermal hysteresis and is a direct measure of antifreeze activity (Kao et al. 1986). In previous studies of adult cod, antifreeze levels have been used in conjunction with details of location and time of capture, to provide evidence of overwintering behavior. Ruzzante et al. (1996*a*) used thermal hysteresis in individual plasma samples to infer the overwintering behavior of the individual cod based on the comprehensive study by Goddard et al. (1994). In adult cod, high antifreeze activity (≥0.2°C thermal hysteresis) is indicative of overwintering in cold (<0°C) water, which was generally inshore, while low or nonexistent winter antifreeze levels (≤0.09°C) are indicative of overwintering in warm water, which was generally offshore.

Genetic techniques

DNA extraction from alcohol-preserved tissue from the farm-held cod samples and from wild cod samples collected between 1992 and 1994 was as described elsewhere (Ruzzante et al. 1996*a*, 1996*b*; Bentzen et al. 1996). Samples collected from wild cod in 1995 were extracted by the following procedure. An aliquot (50–75 µL) of blood–ethanol was diluted in 750 µL of high TE (100 mM Tris–HCL, 40 mM EDTA) spun for 10 s to pellet the cells, and the supernatant was discarded. The cell pellet was then resuspended in 250 µL of MGPL (Marine Gene Probe Laboratory) lysis buffer (10 mM Tris–HCL, 1 mM EDTA, 200 mM LiCl (pH 8.0), and 0.8% SDS), containing 200 µg/mL proteinase K. The sample was incubated at 45°C for 15 min, vortexed, and further incubated until the cells were completely lysed (10–20 min). Following incubation, 500 µL of TE (10 mM Tris–HCl, 1 mM EDTA (pH 8.0)) and 750 µL of cold isopropanol was added and the sample was mixed by vortexing. The DNA was pelleted by pulse spinning of the sample at 14 000 × *g*, and the supernatant was discarded. The sample was then air-dried for 10 min and resuspended in 100 µL of TE. Following resuspension, the sample was diluted to an appropriate concentration for polymerase chain reaction (PCR).

PCR analysis was carried out as described by Brooker et al. (1994) using five dinucleotide microsatellite primers, Gmo2, Gmo132, and Gmo145 (Brooker et al. 1994), Gmo4 (Wright 1993), and Gmo120 (Ruzzante et al. 1996*a*). Gmo2, Gmo4, Gmo120, and Gmo132 are perfect GT repeats, and Gmo145 is a compound $G_x(GA)_x$ repeat as defined by Tautz (1989). The PCR products were resolved on 6.5% denaturing polyacrylamide gels, and the alleles were sized relative to a sequence ladder generated from M13mp18 (Yanisch-Perron et al. 1985).

Sample collections

Wild cod inshore: April 1995

Tissue samples were taken from 100 wild cod collected between 24 and 27 April 1995 in the Smith Sound region of Trinity Bay, Newfoundland (48.18°N, 53.80°W). These samples were used for genetic and antifreeze protein analysis (Table 1). One half (50) of the cod were tagged and released following blood sampling, and the remainder were killed for aging and maturity inspections. These collections were made as part of a Department of Fisheries and Oceans (DFO) research survey trip Shamook-239 using an otter trawl at a nominal depth of 152 m where the bottom temperature was nominally –0.98°C. These wild cod (35 females and 14 males in the sacrificed subsample) ranged from 45 to 85 cm (median 56 cm) fork length and from 0.70 to 5.73 kg (median 1.54 kg) fresh mass representing ages 5–9 years (median 6 years).

Plasma antifreeze levels in these cod were generally lower than those in the farm-held cod (see below). Values of thermal hysteresis ranged from 0.065°C to 0.483°C, and the majority (72%) had values <0.2°C. Previous studies have shown that winter antifreeze levels in adult cod are positively correlated with the length of time spent in subzero water and that wild cod sampled in cold water in Trinity Bay in winter have moderate to high antifreeze levels in their plasma by the beginning of May (Goddard et al. 1994). Thus, the unusual antifreeze data obtained in this study led us to suspect that cod in the April 1995 inshore group had been exposed to two different thermal environments in the recent past: some cod had been resident in cold water for several weeks or months, and some had only recently entered, or returned to, subzero water.

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Table 1. Summary statistics of wild (SH-239, SH-250) and farm-held (Gooseberry Cove rearing pen; FOG) cod samples collected in 1995 in the inshore region of Trintiy Bay, Newfoundland, and used for genetic analysis using microsatellite loci and for comparison with similar samples collected during 1992–1994 and analyzed elsewhere (see table 1, Ruzzante et al. 1996*a*).

Sample identifier	Date	Location	Latitude (°N)	Longitude (°W)	Nominal depth (m)	Nominal water temperature at depth (°C)	*N*	Fork-length range (cm)	Fork-length mean (cm)	Thermal hysteresis range (°C)	Thermal hysteresis mean (°C)
SH-239	April 1995	Smith Sound, Trinity Bay	48.18	53.80	152	–1.0	100	45–85	57.4	0.065–0.483	0.169
SH-250	December 1995	Smith Sound, Trinity Bay	(48.13)[a]	(53.80)	(45)	(2.2)	(50)	(52–65)	(56.9)	—	—
Set-8			48.07	53.73	46	0.2	17	52–65	57.2	—	—
Set-10			48.19	53.87	44	3.3	33	52–64	56.7	—	—
FOG	Collected June 1992, farm held until April 1995	East Random Head and Gooseberry Cove, Trinity Bay	48.05 48.03	53.34 53.65	5	—	30	48–71	64.8	0.167–0.576	0.350

Note: The farm-held cod (FOG) were collected in June 1992 in the area of East Random Head and were moved to Gooseberry Cove were they were pen-reared until April 1995, when they were sampled for this study.

[a]Number in parentheses represents the average of set-8 and set-10.

Wild cod inshore: December 1995

Tissue samples for genetic analysis were drawn from a total of $N = 50$ wild cod caught between 8 and 13 December 1995 in the Smith Sound region of Trinity Bay, Newfoundland (Table 1). Seventeen of these fish were collected during set-8 (48.07°N, 53.73°W) at a nominal depth of 46 m, where water temperature was 0.2°C. The remaining 33 cod were collected during set-10 (48.19°N, 53.87°W) at a nominal depth of 44 m, and the water mass was warmer at 3.3°C. These collections were made as part of a DFO research survey trip Shamook-250 using hand jigs. These wild cod (37 females and 13 males) were generally the same size (and thus, presumably the same age) as the wild cod collected in April and ranged from 52 to 65 cm (median 57 cm) fork length and from 1.28 to 2.49 kg (median 1.65 kg) fresh weight.

Farm-held cod: June 1992 – April 1995

A total of 31 farm-held cod from the Gooseberry Cove, Trinity Bay, Newfoundland, holding pens were collected on 17 April 1995 for genetic and blood antifreeze protein analysis (Table 1). These cod had been originally trapped in June and July of 1992 in the vicinity of East Random Head, Trinity Bay, and had been transferred to a holding pen where they were fed, more or less ad libitum, until sampled as above in 1995. The farm-held cod collection (23 females and 8 males) were on average 10 cm longer and 1 year older than the wild cod (above) and ranged from 48 to 71 cm (median 66 cm) fork length and from 1.75 to 5.20 kg (median 3.48 kg) whole fresh mass representing ages 6–9 years (median 7 years).

Thermal hysteresis values ranged from 0.167 to 0.576°C, and the vast majority exceeded the value of 0.2°C. These values of antifreeze activity reflect recent and prolonged exposure to subzero water temperatures and are consistent with the thermal regime that the cod were exposed to while held in the pen. Further details on the farm-held cod, their growth, etc., are found in Wroblewski et al. (1996).

Wild cod inshore and offshore as reported in Ruzzante et al. (1996a)

Details of the cod sample collections made between January 1992 and June 1994 are found in Ruzzante et al. (1996*a*, their table 1) and are briefly summarized here. Four of the collections were from offshore (North Cape region of the northern Grand Bank) winter and early summer aggregations, and 10 were from winter and early summer aggregations found inshore in the immediate vicinity of the Random Island region of Trinity Bay and elsewhere in Trinity Bay (Bellevue) and in Bonavista Bay. The offshore collections were from depths between 400 and 1000 m, at temperatures of between 2.5 and 3.4°C; the average fork lengths ranged between 33 and 54 cm. Inshore collections of cod were made at depths between 140 and 200 m in the Southwest Arm region of Trinity Bay and at depths of between 14 and 150 m elsewhere inshore.

Average water temperatures at depth associated with collections in the Southwest Arm were consistently subzero (–0.5 to –1.4°C) while collections from other locations, depths, and dates were associated with warmer water (0.6–5.5°C). The average lengths of cod in these inshore collections in the Random Island region ranged between 38 and 62 cm, while those from Bellevue and Bonavista Bay ranged between 27 and 34 cm (juveniles). Those collected from Heart's Ease Ledge in July 1993 were from an aggregation that was known to be spawning at that location (Wroblewski et al. 1996; Smedbol and Wroblewski 1996). Blood plasma thermal hysteresis was determined for 7 of the 10 inshore samples and were in general inversely related to water temperatures (elevated thermal hysteresis at subzero temperatures and depressed or nonexistent thermal hysteresis at temperatures >0°C).

Data analysis

We employed $(\delta\mu)^2$ (Goldstein et al. 1995) and the more recent D_{SW} (Shriver et al. 1995) to estimate genetic distances. Estimates of population structure were obtained using R_{ST} (Slatkin 1995), and for comparative purposes, we also employed the F_{ST} statistic following Weir and Cockerham (1984). Most of these measures were used in our recent studies on Atlantic cod (Bentzen et al. 1996; Ruzzante et al. 1996*a*, 1996*b*). Significance values for all tests (i.e., homogeneity of allele frequency distributions, genetic distances, estimates of population structure) were determined by Monte Carlo simulations and the

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bootstrap method (minimum 1000 resampling trials per individual comparison; Manly 1991). In all cases, bootstrapping was conducted across individuals and populations by reshuffling alleles or genotypes at each locus separately. Bootstrapping each locus independently enables comparison of estimates across loci. Bootstrapping across individuals and populations enables the estimation of significance values for the test under consideration. We assume the five microsatellite DNA loci examined in this study constitute a random sample of the genome. Estimates of R_{ST} and F_{ST} combined over loci were calculated by first averaging numerators and denominators across loci and then taking ratios, as suggested by Weir and Cockerham (1984) and Slatkin (1995).

Results

Variability in microsatellite loci within and among 1995 samples

The three sets of cod collected in 1995 vary in sample size by up to a factor of three (Table 1). Consistent with this difference, the largest sample (SH-239, $N = 100$) had the largest number of private alleles (Table 2). Despite the difference in sample size, however, the total number of alleles present in the smallest sample is only approximately 71% of those present in the largest sample (i.e., 84 vs. 119). As expected for microsatellite loci, observed (H_{obs}) and expected heterozygosities (H_{exp}) were high and varied between 0.877 and 0.942 and 0.852 and 0.870, respectively.

Single-locus analysis for the combined data ($N = 180$) indicates that, again as expected, the three loci with the highest number of alleles (i.e., Gmo4, Gmo120, and Gmo145) were also the loci with the highest observed and expected heterozygosities (Table 3). There was no evidence for deficiency or excess of heterozygotes as measured by $d = (H_{obs} - H_{exp})/H_{exp}$ over all loci ($d = 0.0107$, $P = 0.337$; Table 3).

In the following sections we (*i*) analyse the two wild cod samples collected inshore in 1995, (*ii*) compare them to inshore and offshore samples collected between 1992 and 1994 and reported in Ruzzante et al. (1996*a*), and (*iii*) compare the farm-held cod originally captured in 1992 with various subsets of the 1995 wild inshore samples and with the group of offshore cod.

(*i*) Comparison between wild cod samples collected inshore in 1995

Comparison of the samples collected inshore in April and December 1995 indicated a marginal degree of genetic difference between them when all individuals in both samples were considered ($((\delta\mu)^2 = 4.17$, $P = 0.07$; $D_{SW} = 0.031$, $P = 0.044$, $N_1 = 100$, $N_2 = 50$; Table 4).

This result led us to consider the potential heterogeneity within each of the April and December 1995 samples using information on antifreeze activity, as well as water temperature at the sampling depth. We first distinguished among individuals from the April 1995 collection according to whether they exhibited high (>0.20°C) or low (<0.09°C) thermal hysteresis (level of antifreeze activity) as detailed in Ruzzante et al. (1996*a*). We similarly distinguished among individuals from the December 1995 collection on the basis of water temperature at sampling depth. There was no significant difference between the subset collected in April 1995 that showed high thermal hysteresis (>0.20°C, $N = 29$) and that subset collected in the same month that showed low (<0.09°C, $N = 31$) thermal hysteresis ($(\delta\mu)^2 = 0.373$, $P = 0.948$; $D_{SW} = -0.0507$). Similarly, there was no significant difference between the subset of fish collected in April 1995 that showed high thermal hysteresis (>0.20°C, $N = 29$) and that subset of fish from the December 1995 collection from relatively cold water (0.2°C, set-8, $N = 17$; Table 1) using either $(\delta\mu)^2$ (0.897, $P = 0.92$) or D_{SW} (–0.024). We also found no evidence of population structure in contrasts between those cod showing high antifreeze activity (April 1995) and those sampled in relatively warm (3.3°C) water in December (set-10, $N = 33$; Table 1) nor between those fish with low antifreeze activity (April 1995, $N = 31$) and cod sampled in relatively cold water in December (set-8, $N = 17$; Table 1). We did find that cod with low antifreeze activity in April ($N = 31$) differed significantly from cod sampled in relatively warm water in December ($N = 33$). The results of these contrasts, coupled with the heterogeneity detected above when all fish in both samples (i.e., April and December 1995) were considered, suggest that some of these subsets, or perhaps all of them, may be constituted by cod of heterogeneous origin, an issue we address later in the Discussion. It should be noted that sample sizes among these secondary comparisons are low, and frequently unequal, making it unlikely for statistically significant genetic distances to be detected when true distances may be small (Ruzzante 1998).

To summarize thus far, both $(\delta\mu)^2$ and D_{SW} provided evidence of weak differences between the two wild inshore samples collected in 1995, but these differences did not appear to be correlated with antifreeze activity nor with water temperature at the depth of sampling. In the following analysis we ignore this potential heterogeneity and pool the two sets of inshore data collected in 1995, hereafter referred to as IN-95, and proceed to compare them with data from the inshore and offshore samples collected between 1992 and 1994 and analyzed elsewhere (Ruzzante et al. 1996*a*).

(*ii*) Comparison of 1995 wild inshore cod with 1992–1994 wild inshore and offshore cod

The IN-95 pool ($N = 150$) did not differ from the 1992–1994 cod collected inshore in cold water with high (>0.20°C) antifreeze activity (inshore overwintering) ($(\delta\mu)^2 = 1.26$, $P = 0.240$; $D_{SW} = 0.0039$, $P = 0.270$; $R_{ST} = 0.028$, $P = 0.217$; Table 4, but see F_{ST} result). However, it did differ significantly from the 1992–1994 cod collected offshore during winter with three of the four genetic measures ($(\delta\mu)^2 = 2.096$, $P = 0.047$; $D_{SW} = 0.0192$, $P = 0.021$; and $F_{ST} = 0.0014$, $P = 0.0049$; Table 4). There was no statistical evidence with any of the four measures that the 1995 inshore pool differed from low (<0.09°C) antifreeze cod collected between 1992 and 1994 from warm inshore water in summer (Table 4).

The estimates for $(\delta\mu)^2$ and D_{SW} (but not those for R_{ST} and F_{ST}, see Discussion) were 2- to 5- and 10-fold larger, respectively, in the comparison of IN-95 with offshore cod than they were for comparisons involving the different inshore pools (Table 4). Thus, despite the internal heterogeneity detected within the pool of cod collected inshore in April and December 1995, these cod, collectively, were different from offshore overwintering cod and were indistinguishable from cod that had been collected inshore at different times of the year between 1992 and 1994 and that showed either high or low antifreeze activity.

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Table 2. Degree of genetic variation (sample size: *N* (indiv.), mean sample size per locus, number of private alleles, total number of alleles, mean number of alleles per locus, mean observed (H_{obs}) and expected (H_{exp}) heterozygosity, and mean heterozygote deficiency (*d*)) among three samples of northern cod (*Gadus morhua*) at five microsatellite loci and collected from the inshore regions of Trinity Bay, Newfoundland, in 1995: Shamook-239, April 1995 (SH-239); Shamook-250, December 1995 (SH-250); and farm-held cod from the Gooseberry Cove rearing pen (FOG).

Sample	*N* (indiv.)	Mean sample size per locus	No. of private alleles	Total no. of alleles	Mean no. of alleles per locus	Mean H_{obs}	Mean H_{exp}	Mean *d*
April 1995	100	97.4	23	119	23.8	0.877	0.870	0.0058
December 1995	50	49.4	9	98	19.6	0.862	0.852	0.0022
FOG	30	28.2	6	84	16.8	0.942	0.870	0.0877

(*iii*) Comparison of farm-held cod (captured in 1992) with wild cod inshore

We now consider the farm-held cod ($N = 30$) originally captured inshore in 1992 in relation to various subsets of wild cod collected inshore in 1995. Estimates of both $(\delta\mu)^2$ and D_{SW} indicate we cannot reject the hyptohesis that the farm-held cod were drawn from the same population of inshore wild cod showing high (>0.20°C) thermal hysteresis in April 1995 ($N = 29$) nor from the same population of cod collected inshore in relatively cold (0.2°C) water in December 1995 ($N = 17$), even when the latter two subsets were combined ($N = 46$) and the analysis was repeated (Table 4). The estimates of genetic distance between the farm-held cod and wild cod collected inshore in April 1995 and showing low thermal hysteresis (<0.093°C, $N = 31$) were twofold larger than in the previous set of comparisons, but they were not significant ($P \geq 0.15$). The estimates of genetic distance between the farm-held cod and the wild cod collected inshore in December 1995 in relatively warm water ($N = 33$) were sixfold larger than in the first set of comparisons and were significant or marginally so (Table 4). Thus, the farm-held cod were more different from the low-antifreeze cod from April 1995 than they were from the high-antifreeze cod also from April 1995, and they were significantly different from the cod collected in December 1995 in warm water. In addition, the farm-held cod were significantly different from the pool of offshore cod ($(\delta\mu)^2 = 8.10$, $P = 0.025$; $D_{SW} = 0.054$, $P = 0.029$; Table 4, line 10). However, interpretation of these results may be difficult because of the difference in sample size between these two groups (Ruzzante 1998). We therefore pooled the farm-held cod with the group of wild cod collected inshore in 1995 and compared this group (hereafter IN-95 + FOG; $N = 180$) against the offshore cod ($N = 140$). The pool of inshore and farm-held cod differed genetically from the offshore cod with all four measures used ($(\delta\mu)^2 = 2.32$, $P = 0.031$; $D_{SW} = 0.0188$, $P = 0.018$; $R_{ST} = 0.0016$, $P = 0.078$; $F_{ST} = 0.0015$, $P = 0.030$), and all estimates were of the same magnitude as (if not slightly larger than) when the farm-held cod were not included in the inshore pool (cf. Table 4, lines 4 and 11).

Comparison of farm-held cod with wild, inshore cod from Bellevue and Hearts Ease Ledge

Estimates of genetic distance are relatively large and significant for the comparison between the farm-held fish ($N = 30$) and fish collected more or less simultaneously in July 1992 in the area of Bellevue, Trinity Bay ($N = 26$; $(\delta\mu)^2 = 17.62$, $P = 0.004$; $D_{SW} = 0.1240$, $P = 0.013$; Table 4, line 12). In contrast, no differences were found between the farm-held fish and fish collected in July 1993 in the area of Heart's Ease Ledge, Trinity Bay ($N = 25$), from an aggregation that was known to be spawning at the time ($(\delta\mu)^2 = 4.47$, $P = 0.355$; $D_{SW} = 0.0259$, $P = 0.252$; Table 4, line 13).

Discussion

We have shown, on the basis of microsatellite DNA variation, that cod sampled inshore in April of 1995, and later in December of 1995 in the area of Smith Sound, western Trinity Bay, were collectively genetically indistinguishable from cod populations overwintering inshore in the same general area during 1992–1994 and were genetically distinguishable from cod populations overwintering offshore along the edge of the contintental shelf on the Grand Bank during 1992–1994. These are consistent with very similar results reported in Ruzzante et al. (1996*a*) and, therefore, provide evidence of temporal stability in the genetic differences between inshore and offshore overwintering cod populations in the region, at least at the scale of 2–3 years. We have further shown that a group of farm-held cod, initially caught inshore in western Trinity Bay in the summer of 1992 and subsequently pen reared for 3 years, were also representative of the inshore overwintering population from which they were presumably drawn originally.

Virtually all of the genetic measures employed above led us to statistically reject the hypothesis of no genetic structure between inshore and offshore overwintering cod. Differences between the complex of wild inshore cod collected in 1995 and that offshore collected between 1992 and 1994 was detected with the $(\delta\mu)^2$ and D_{SW} estimates of genetic distance, as well as with the F_{ST} estimate of population structure (Table 4). Although these results provide evidence of temporal stability in the genetic structure within the northern cod complex overwintering inshore and offshore, they represent only a partial test of temporal stability as no wild cod samples from the northern Grand Bank region (i.e., offshore) were available in 1995. A more rigorous test of the temporal stability hypothesis would require the collection and processing of more or less contemporaneous offshore and inshore samples, the former now being difficult to obtain. What is of particular interest in this study, which is in contrast to the results reported in Ruzzante et al. (1996*a*), is that the genetic differences detected here between inshore and offshore pools were not as clearly associated with antifreeze activity (thermal hysteresis) among the cod sampled inshore. Antifreeze appears in the plasma of adult cod as a direct result of prolonged residence in subzero water. In previous studies, cod sampled in winter in Trinity Bay have had high antifreeze levels indicating long-term residence in subzero (inshore) water (most likely the Trinity Bay area; Goddard

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Table 3. Single-locus statistics (sample size, *N*; number of alleles, *n*; allele size range in base pairs; observed and expected heterozygosity, H_{obs}, H_{exp}; and heterozygote defficiency (*d*)) for each of five microsattelite cod loci among three sample sets of northern cod (*Gadus morhua*) collected from the inshore regions of Trinity Bay, Newfoundland, in 1995.

Locus	*N*	*n*	Size range	H_{obs}	H_{exp}	*d*
Gmo2	179	19	104–148	0.782	0.779	0.0047
Gmo4	171	37	115–265	0.994	0.953	0.0437
Gmo120	177	31	152–222	0.983	0.944	0.0415
Gmo132	172	10	105–135	0.698	0.729	–0.0427
Gmo145	176	42	147–207	0.955	0.928	0.0065
Mean over all loci				0.882	0.870	0.0107 ($P = 0.337$)

et al. 1994). In the present study antifreeze levels in cod sampled inshore in winter were remarkably heterogeneous suggesting exposure to thermal regimes with temperatures >0°C (antifreeze low or absent) and <0°C (antifreeze high). The absence of a tight correlation between antifreeze level and genetic difference in this study may thus reflect movement of cod among thermal regimes inshore. Alternatively, this absence of a correlation may be a result of the relatively small sample sizes for the different categories of antifreeze levels used in the present study relative to the previous study. Whatever the reason, the results clearly indicate that, regardless of antifreeze levels, cod sampled inshore in April and December 1995 were genetically distinguishable from offshore cod populations and were genetically indistinguishable from inshore cod populations collected between 1992 and 1994.

The subset of farm-held cod collected in 1992 was statistically most likely to have been drawn from an inshore overwintering population: (*i*) they were statistically indistinguishable from wild inshore cod with high antifreeze activity collected in April 1995; (*ii*) they were likewise indistinguishable from cod collected in relatively cold water in December 1995; and (*iii*) although they were indistinguishable from cod with low antifreeze activity collected in April 1995 (Table 4, line 8), they were different (with $(\delta\mu)^2$ and marginally so with D_{SW}; Table 4, line 9) from cod collected in relatively warm water in December 1995. These findings suggest that the April and December 1995 inshore cod that appeared to have a preference for warmer overwintering environments may be genetically farther removed from the more homogeneous (farm-held) inshore cod than those April and December 1995 cod collected inshore in cold water with high antifreeze levels. Furthermore, either by themselves, or grouped with the wild cod collected inshore in 1995, the farm-held cod were genetically different from offshore cod (Table 4, lines 10 and 11).

Our results indicate that the genetic differences detected earlier (Ruzzante et al. 1996*a*) between cod that overwinter inshore in the area of western Trinity Bay and cod that overwinter offshore in the area of the Grand Bank are temporally stable, at least at the scale of 2–3 years. However, it is also important to ask whether the magnitude of the differences remained more or less constant over the period 1992–1995. A comparison of the $(\delta\mu)^2$ estimate for the inshore 1995 pool versus offshore pool in the present study (Table 4, line 4) with that in our earlier study (Table 4, line 1; Ruzzante et al. 1996*a*) shows that the genetic distance between inshore and offshore pools remained virtually unchanged (i.e., 2.10 vs. 2.17 respectively). Ruzzante et al. (1996*a*) did not use D_{SW}, but values for the different contrasts of the 1992–1994 inshore and offshore pools using this measure are reported here in a footnote to Table 4. Again, the D_{SW} estimate for the comparison between the 1995 inshore pool and offshore cod (Table 4, line 4) is of a similar magnitude to that between the inshore and offshore 1992–1994 pools (i.e., 0.0192 vs. 0.0175, respectively). The R_{ST} and F_{ST} estimates for the 1992–1994 inshore–offshore contrast without discrimination of the inshore fish according to antifreeze activity were not reported in Ruzzante et al. (1996*a*). We calculated these estimates as $R_{ST} = 0.0013$ and $F_{ST} = 0.0007$, which are similar to (in the case of R_{ST}), or smaller than (in the case of F_{ST}) the respective values reported in this study (i.e., $R_{ST} = 0.0012$ and $F_{ST} = 0.0014$; Table 4). We conclude that not only did the structure detected earlier (Ruzzante et al. 1996*a*) between inshore overwintering cod from Trinity Bay and offshore overwintering cod from the Grand Bank remain in existence, but the magnitude of the genetic distance between these groups, however measured, has remained virtually identical.

The issue of temporal stability in genetic structure has previously been considered for a number of marine species using a variety of genetic techniques and, not surpringly, conclusions vary with the species under consideration. For example, using mitochondrial DNA haplotype frequencies, Brown et al. (1996) recently demonstrated that, for American shad (*Alosa sapidissiima*), there is relatively little temporal variation compared with the existing geographically based variation among populations from different river drainages spanning the distributional range of the species. On the other hand, allozyme genotypic frequencies in Pacific salmon populations can differ markedly over time, in part because of the unique life-history features of salmonids, and single "snapshot samples" are unlikely to adequately define stocks (Waples 1990; Waples and Teel 1990). Therefore, pooling of periodic samples to average out any temporal changes in allele frequencies has been suggested for analyses of mixed-stock fisheries in these species (Waples1990; Waples and Teel 1990; see also Brown et al. 1996). Significant temporal genetic variation has also been found among populations of queen conch (*Strombus gigas*) in Florida Keys (Campton et al. 1992). For some species, the degree of temporal variation in allele frequencies has been shown to differ among life-history stages. For example, using allozymes, Kordos and Burton (1993) compared the temporal variation in allelic frequencies among larvae, juveniles, and adults of the blue crab (*Callinectes sapidus*). They detected seasonal variation in larval allelic frequencies, with summer samples being more variable than winter samples, which

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Table 4. Estimates, with *P* values given in parentheses, of $(\delta\mu)^2$, D_{SW}, R_{ST}, and F_{ST}.

Sample comparison	N_1	N_2	$(\delta\mu)^2$	D_{SW}*	R_{ST}	F_{ST}
(1) Apr.-95 vs. Dec.-95	100	50	4.17 (0.070)	0.0310 (0.044)		
(2) IN-95 vs. IN92–94-HI-AF	150	123	1.26 (0.240)	0.0039 (0.270)	0.028 (0.217)	0.0030 (0.059)
(3) IN-95 vs. IN92–94-LO-AF	150	58	0.28 (0.933)	–0.01127 (NA)	0.035(0.229)	0.0006 (0.279)
(4) IN-95 vs. OFFSHORE	150	140	2.10 (0.047)**	0.0192 (0.021)	0.0012(0.250)	0.0014 (0.049)
(5) FOG vs. Apr.–95-HI-AF	30	29	3.58 (0.418)	0.017 (0.262)		
(6) FOG vs. Dec.-95-COLD	30	17	2.48 (0.650)	–0.018 (NA)		
(7) FOG vs. Apr.-95-HI-AF + Dec-95-COLD	30	46	2.95 (0.440)	0.0092 (0.311)		
(8) FOG vs. Apr.-95-LO-AF	30	30	5.13 (0.258)	0.0397 (0.155)		
(9) FOG vs. Dec.-95-WARM	30	33	12.25 (0.010)	0.0656 (0.076)		
(10) FOG vs. OFFSHORE	30	140	8.10 (0.025)	0.054 (0.029)		
(11) IN-95 + FOG vs. OFFSHORE	180	140	2.32 (0.031)	0.0188 (0.018)	0.0016 (0.078)	0.0015 (0.030)
(12) FOG vs. Bellevue	30	26	17.62 (0.004)	0.1240 (<0.013)		
(13) FOG vs. HEL	30	25	4.47 (0.355)	0.0259 (0.252)		

Note: Comparisons between samples (N_1, N_2) collected in inshore areas nearby Trinity Bay, Newfoundland, in April and December 1995, and samples collected offshore and inshore between 1992 and 1994 and reported in Ruzzante et al. 1996*a*. Comparisons of the magnitude of estimates across pairwise contrasts are valid only across contrasts with roughly equivalent sample sizes (see Ruzzante 1998). Apr.-95-HI-AF and Apr.-95-LO-AF, subsets from April 1995 with high and low antifreeze, respectively; Dec.-95-COLD and Dec.-95-WARM, subsets from December 1995 from relatively cold and relatively warm water, respectively; IN-95, April 1995 and December 1995 samples pooled; IN92–94-HI-AF and IN92–94-LO-AF, subsets from the inshore collections from 1992 to 1994 (reported in Ruzzante et al. (1996*a*)) showing high and low antifreeze levels respectively; FOG, farm-held cod from the Gooseberry Cove rearing pen; HEL, cod from Hearts Ease Ledge reported in Ruzzante et al. (1996*a*), as were Bellevue cod.

*D_{SW} estimates between inshore and offshore 1992–1994 pools were not reported in Ruzzante et al. (1996*a*). D_{SW} estimates (*P* values) for comparisons between those pools are as follows: IN92–94 vs. OFFSHORE: 0.0175 ($P = 0.016$); IN92–94-HI-AF vs. OFFSHORE: 0.0240 ($P = 0.015$); IN92–94-LO-AF vs. OFFSHORE: –0.0003 (NA); IN92–94-HI-AF vs. IN92–94-LO-AF: –0.0073 (NA).

**A very similar result (i.e., 2.17) was reported in Ruzzante et al. (1996*a*).

showed relative homogeneity in allele frequencies. The authors attributed these changes to seasonal differences in population sources (Kordos and Burton 1993).

Despite the evidence for the temporal stability we have provided and the nature of variation that might be expected based on other studies, some inconsistencies among our results require explanation. For example, on the one hand there were clear genetic affinities and differences between the farm-held cod and the various subsets of wild inshore cod (e.g., high vs. low antifreeze activity, etc.), while on the other hand there was evidence of genetic heterogeneity within the pool of wild cod collected inshore in 1995, which was neither clearly associated with antifreeze activity nor with water temperature at sampling depth. Despite this heterogeneity, when the 1995 inshore cod were considered as a group they were different from offshore cod. We interpret these results to suggest that the subset of farm-held cod was comprised of fish that were genetically more homogeneous than any of the subsets of wild cod collected in 1995. This suggestion is supported by the fact that, while the farm-held cod were genetically different from cod sampled more or less simultaneously in June of 1992 in the Bellevue area of Trinity Bay (but see below), they were genetically indistinguishable from spawning cod sampled in July 1993 in the immediate vicinity (Hearts Ease Ledge) of their original capture (see Wroblewski et al. 1996). Considered collectively, these observations and results lead us to conclude that the pool of inshore cod is comprised of weakly heterogeneous subsets of inshore cod, which as a group, are distinguishable from offshore cod. Alternatively, the weak heterogeneity detected within the inshore samples collected in 1995 may be a result of what appears to be a very recent (1995 and 1996 following the collapse of northern cod) anomaly in the normal pattern of cod migration, whereby large aggregations of cod that are normally found offshore are no longer found there (Shelton et al. 1996) and may be remaining inshore (see Brattey 1996; Lilly 1996; Rose 1996; Taggart 1996). These large aggregations apppear to be in addition to what are normally inshore overwintering and reproducing populations (see review in Lilly 1996). Nevertheless, despite the internal heterogeneity within the inshore pool of cod we analyzed, this pool remains genetically different from what were known to be offshore cod, at least up until 1994.

The above discussion raises an intriguing question that remains unanswered. We ask, to what extent can different inshore cod aggregations or samples from different, though neighbouring, regions inshore be considered as genetically homogeneous, especially in relation to offshore overwintering cod populations. For example, the farm-held cod were similar to cod from a large spawning aggregation collected 1 year later, in July 1993, in the area of East Random Head but were different from cod collected at the same time, in June 1992, in the area of Bellevue. Note, however, that this last group (Bellevue) was comprised entirely of juveniles (length 27.1 ± 3.9 cm (mean $\pm$ SD), $N = 26$; see Ruzzante et al. 1996*a*, Table 1). Thus, the possibility that they may have originated offshore, may have drifted inshore as eggs or larvae (Davidson and DeYoung 1995), and are too young to be moving back to the offshore spawning grounds cannot be discounted. Such a scenario is supported by the fact that the juvenile cod sampled in Bellevue ($N = 26$) appear genetically indistinguishable from the pool of offshore cod collected between 1992 and 1994 ($N = 140$, $(\delta\mu)^2 = 3.81$, $P = 0.197$; $D_{SW} = 0.0008$). Whatever the origin of the juvenile cod sampled in Bellevue, the various comparisons within the group of cod collected inshore in April and December 1995 suggest that cod from different inshore aggregations or from neighbouring inshore regions may be

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genetically heterogeneous and may thus be reproductively isolated, or at least partially so.

Bentzen et al. (1996, p. 2719) suggested that "... it may be wise to seriously reconsider the current (and historical) management scheme for northern cod, which presumes one stock distributed throughout the inshore and offshore regions stretching from Hamilton Bank to the Grand Bank through NAFO divisions 2J, 3K, and 3L." That suggestion was based primarily on a north–south population structure of offshore northern cod. Our results here further suggest a reconsideration based on what appears to be an inshore–offshore (east–west) structure. The nature of such reconsideration is entirely consistent with Templeman (1962, p. 108) who envisioned that: "Very likely in the future enough differences will be found to indicate a number of north–south and inshore–offshore substocks of this Labrador–Newfoundland stock which either do not intermingle greatly or separate out at certain seasons." From a geographic perspective, the concept of inshore statistical and management units independent of offshore units at least in Division 3L is not new (Halliday and Pinhorn 1990, Figs. 10 and 11, pp. 16 and 17) and might be useful in any management reconsideration, especially given that it is not clear from where "rebuilding" of the northern cod stock complex might stem: inshore, offshore, or both. The feasibility of such a management approach is equally unclear.

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PERSPECTIVE

Development of research on fish behaviour as part of fisheries science in Canada

Miles H.A. Keenleyside

Abstract: Fisheries research in Canada had roots in the late nineteenth century, developed gradually until after the Second World War, and then expanded rapidly. The scientific study of animal behaviour also originated in the late 1800s, but in Canada the field of fish behaviour was of minor interest until the late 1940s, when F.E.J. Fry and W.S. Hoar established influential research programs. Hoar, in particular, was influenced by ethology, an approach to animal behaviour pioneered in the 1930s and 1940s in Europe by Karl von Frisch, Konrad Lorenz, and Niko Tinbergen. As the field of behaviour expanded and diversified, behavioural ecology became one of its most dynamic branches. Canadian fish behaviourists have actively contributed to this development. Analysis of publications in the *Canadian Journal of Fisheries and Aquatic Sciences*, the *Canadian Journal of Zoology*, and six leading journals of animal behaviour confirm these trends. Most Canadian fish behaviour research was based on salmonids until the mid-1960s, after which there was much greater diversity in study species. Migration, orientation, and reactions to environmental stimuli were the problems most actively studied by Canadian fish behaviourists before 1965. Since then, reproductive and other social behaviour patterns, feeding, and predator avoidance have become areas of major interest.

Résumé : Au Canada, la recherche dans le domaine des pêches a pris naissance à la fin du XIX^{e} siècle, a progressé lentement jusqu'à la fin de la Deuxième Guerre mondiale, puis s'est développée rapidement par la suite. L'étude scientifique du comportement animal a aussi vu le jour à la fin du siècle dernier, mais, au Canada, le domaine du comportement des poissons a été peu étudié jusqu'à la fin des années 1940, au moment où F.E.J. Fry et W.S. Hoar ont élaboré des programmes de recherche qui allaient prendre une grande importance. Hoar, en particulier, a été influencé par l'éthologie, approche de l'étude du comportement animal lancée dans les années 1930 et 1940 en Europe par Karl von Frisch, Konrad Lorenz et Niko Tinbergen. Le champ de l'étude comportementale s'est étendu et diversifié, l'écologie comportementale devenant l'un de ses secteurs les plus dynamiques. Les chercheurs canadiens dans le domaine du comportement des poissons ont contribué activement à rendre ce secteur dynamique; l'analyse de publications parues dans le *Journal canadien des sciences halieutiques et aquatiques*, dans la *Revue canadienne de zoologie* et dans six autres publications de premier plan sur le comportement animal en fait foi. Au Canada, la majorité des recherches sur le comportement des poissons portait sur les salmonidés jusqu'au milieu des années 1960; ensuite, les études se sont diversifiées. Avant 1965, les comportementalistes canadiens travaillaient surtout sur les questions de migration, d'orientation et de réactions aux stimuli environnementaux. Depuis lors, les comportements génésiques et autres comportements sociaux, l'alimentation et l'évitement des prédateurs sont devenus des secteurs très étudiés.
[Traduit par la Rédaction]

Introduction

Fisheries research in Canada dates from the late nineteenth century. It began as a result of the curiosity of a few individuals, including naturalists, fishermen, and university professors, but soon expanded as the need arose to solve problems associated with the commercial fisheries (Prince 1920). The scientific study of animal behaviour, including that of fishes, began at about the same time. However, most of the pioneering behaviourists were based in Europe and the United States (Dewsbury 1989); the development of animal behaviour as an active field for scientific research in Canada did not occur until the mid-twentieth century. The main purpose of this paper is to describe the origin and early development in Canada of research in fisheries science in general and fish behaviour in particular. It is not a detailed, comprehensive history of these fields; rather, it is a brief description of the origins of Canadian fisheries science, with emphasis on the role of the federal government in its early growth. In addition, I describe the growth in Canada of research in fish behaviour, which occurred as the study of animal behaviour in general became established as a productive field of research in this country.

My interest in this subject stems from my early experience in zoology. As an undergraduate student in the early 1950s at the University of British Columbia, I was introduced to the field of animal behaviour by William S. Hoar, a pioneer investigator of the physiology and behaviour of fishes. I then did graduate work with Gerard P. Baerends at the University of Groningen, Holland. His laboratory was one of the early

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centres of animal behaviour research in Europe. At about that time, ethology, a new approach to the study of animal behaviour, was expanding from its origins in Germany and Holland into Britain and North America. Also, the Canadian scientific community, both in governments and universities, was rapidly growing, mainly as a result of the increasing prosperity of Canadian society and a widespread optimism about the values of higher education and scientific research in the post-Second World War period. Thus, I was lucky to be involved in the early stages of a new approach to research in animal behaviour at a time when science in Canada was entering a period of vigorous growth.

My thesis in this paper is that while population biology, in the broadest sense, has for a long time dominated fisheries science, the study of behaviour (including both the direct observation and study of fishes in nature and controlled laboratory experimentation) has added a new perspective to the attempts to understand the interactions between fishes and their environment, including the influence of human activities. To add some quantitative strength to this thesis, I present data on the frequency of publication in fisheries and fish behaviour and also on the types of fishes most often used and the topics most commonly studied in Canadian fish behaviour research.

Early development of fisheries research in Canada

The capture and processing of fish for human consumption could be described as Canada's oldest industry. A fishery for Atlantic cod (*Gadus morhua*) off the Atlantic coast began almost exactly 500 years ago, after John Cabot returned to England from his first voyage of discovery in 1497. His description of cod abundance off what is now Newfoundland was "that the sea there is swarming with fish, which can be taken not only with the net but in baskets let down with a stone, so that it sinks in the water" (Innes 1954, p. 11). On the Pacific coast, discovery and exploration by Europeans occurred about 300 years later and commercial fishing, based primarily on salmon (*Oncorhynchus* spp.), did not begin until the second half of the nineteenth century (Johnstone 1977).

Of course, an interest in and dependence on fishes as food in Canada has a much longer history than this. Before European settlement began in North America, aboriginal peoples had been living here for thousands of years. Those living near coastal waters or the larger lakes and rivers were dependent on freshwater or anadromous fishes for essential food supplies (Rostlund 1952).

However, the first attempts at organized, scientific investigation of Canada's fishes did not begin until the late 1800s. These were sporadic efforts, often by university professors during their summer break from classes, conducted at various freshwater and marine locations. They gradually accumulated basic information on species' identity, distribution, and some life history features, such as diet, predators and parasites, breeding season, etc. Virtually no experimental work was done on the physiology, ecology, or behaviour of even the most common fishes (Dymond 1964; Johnstone 1977).

Then, towards the end of the nineteenth century, the commercial fishing industries, especially on the east and west coasts of Canada, began to suffer from problems that could not be solved by the fishing companies or the fishermen themselves. Scientific advice was apparently needed and the federal government came under increasing pressure to provide it. In response, E.E. Prince, a fish embryologist from Scotland, was appointed in 1893 as Commissioner and General Inspector of Fisheries in the federal Department of Marine and Fisheries. He promptly began gathering support for the establishment of a government-financed marine biological station to serve as a base for scientific research on fishes and marine invertebrates. There was already considerable interest in the idea, on the part of the fishing industry, university-based biologists (some with experience at marine laboratories in England, Scotland, Italy, and the United States), and also the Royal Society of Canada (Johnstone 1977).

In 1898 the federal government established the Marine Biological Station of Canada to be administered by a Board of Management, with E.E. Prince as director. The first Board had nine members, eight of whom were professors from universities that supported the concept of a marine research station. The purpose of the station was twofold: to provide useful information for the fishing industry and to accumulate basic knowledge about the important species. Thus, Canada's first marine biological laboratory was designed from the start as a place where scientists could conduct both basic and applied research.

The early history of the Marine Biological Station has been colourfully described by Johnstone (1977). The first major problem was to choose a suitable site. After much debate, no doubt including lobbying by politicians, businesspeople, and others interested in having it in their own community, it was decided to build a mobile laboratory that could be moved by scow from place to place on the east coast. For the first 2 years, 1899 and 1900, it was based at St. Andrews, New Brunswick. It was moved to Canso, Nova Scotia, for 1901–1902, to Malpeque, Prince Edward Island, for 1903–1904, and to Gaspé, Quebec, for 1905–1906. In 1907, while it was being towed to Sept-Îles, Quebec, the scow developed serious leaks. The building was beached at Grand Vallée and later abandoned.

To replace the mobile laboratory, the first permanent biological station was built at St. Andrews in 1908. In the same year, because of similar pressures from industry and scientists, the federal government built another research station at Nanaimo, British Columbia, for the study of the fishes and other marine life of the west coast. Meanwhile, in 1901 the government had provided funds to build a biological station at Go Home Bay on Georgian Bay, Ontario. Its purpose was to investigate the aquatic fauna and flora of that region, with emphasis from the start on bass (*Micropterus*). At first, it was controlled by the Madawaska Club, which had been formed at the University of Toronto to create a university summer settlement. In 1904, control passed to the federal Department of Marine and Fisheries. Most of the scientific work at the Go Home Bay Station was done by scientists from the University of Toronto. Among them were two men who later made major contributions to fisheries research and administration in Canada. W.A. Clemens, who spent the summer of 1912 there, was director of the Pacific Biological Station at Nanaimo from 1924 to 1940. A.G. Huntsman was at Go Home Bay for several summers between 1904 and 1910, later spent time at the biological stations in Nanaimo and St. Andrews, and was director of the latter from 1919 to 1934. Huntsman was probably the

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single most influential scientist in the early development of fisheries research at the federal government level in Canada. In addition to his long association with the station at St. Andrews, he was chief editor of research publications of the federal Department of Fisheries from 1934 to 1949. He was also a faculty member at the University of Toronto from 1907 to 1954, and many of his graduate students went on to successful research careers in government service and universities. In 1969 the Huntsman Marine Laboratory (now the Huntsman Marine Science Centre) was established in his honour in St. Andrews, on land adjacent to the biological station. It is a marine research and education centre, supported by governments, private funds, and several universities.

From 1901 to 1924, research at the government biological stations was done mainly by volunteer investigators, who came from universities and museums for the summer months, often with their students. Even the directors were there only in the summers, spending the 8-month academic years at their home universities. The volunteers worked during the summers without salary, but usually with travel expenses and accommodation at the station provided (Clemens 1958). Beginning in 1925, as the demand for the solution of fishing industry problems increased, full-time research scientists were appointed to conduct this work. Until 1934, volunteer seasonal workers and permanent staff scientists worked together to the benefit of both (Hart 1958; Needler 1958). Then, in the depths of the economic depression in 1934, the volunteer support system was suspended for lack of adequate funds. Fisheries research continued at a modest level during the war years of 1939–1945, with emphasis on increasing yields of the fisheries as an important source of protein. After 1945, in response to increasing demands for research on domestic and international fisheries problems, there was a steady increase in personnel and facilities (Hart 1958; Needler 1958).

In summary, through the first half of the twentieth century, fisheries research in Canada grew from modest beginnings at small marine stations at St. Andrews and Nanaimo, staffed by volunteer investigators, to large and expanding facilities at several locations, with full-time scientific and technical personnel, supported by well-equipped laboratories and research vessels. Both basic and applied research was encouraged from the start, but as the financial commitment from the federal government grew, so did the emphasis on solving problems of the commercial fisheries (Ricker 1975). The role of studies on fish behaviour as part of this expanding and increasingly sophisticated fisheries research scene in Canada will now be explored.

Early development of research on animal behaviour

The beginnings of a scientific approach to the study of animal behaviour can be found in the second half of the nineteenth century (Klopfer 1974; Thorpe 1979; Dewsbury 1989). The most influential figure in this development was Charles Darwin. His books "The descent of man and selection in relation to sex" (Darwin 1871) and "The expression of the emotions in man and animals" (Darwin 1872) included descriptive detail on the behaviour of a wide range of invertebrate and vertebrate animals. In addition, Darwin proposed a general explanation for this enormous variability through the mechanism of natural selection, which he had presented earlier in "On the origin of species" (Darwin 1859).

Presumably, an active interest in understanding the behaviour of animals in nature has a much longer history than this. There is no reason to doubt that since the origin of *Homo sapiens* on the earth, humans have known a lot about the animals on which they depended for food and those that were their important predators and competitors (Schneirla 1958). Even today, animal behaviourists can learn much from the observations and understandings of naturalists, pet owners, farmers, hunters, and fishermen. However, a truly scientific approach to understanding animal behaviour did not emerge until the late nineteenth century, and Darwin was the key figure in that development.

Among Darwin's strongest supporters was his friend G.J. Romanes, who was a pioneer in invertebrate physiology and, in the heady intellectual climate of early post-Darwinism, expanded his interests to include animal behaviour, and especially to compare intelligence and the evolution of the mind in higher animals and humans (Thorpe 1979). However, in his books "Animal intelligence" (Romanes 1882) and "Mental evolution in animals" (Romanes 1883), he often depended on anectodal, subjective evidence and a fuzzy concept of "instinct" to explain behavour. The often extreme anthropomorphism of Romanes and some of his contemporaries aroused strong opposition. In particular, the influential British psychologist C. Lloyd Morgan urged students of animal behaviour to strive for objectivity, to develop operational definitions for terms, to use replication in experiments, and to restrict the term "instinct" to species-specific behaviour that is congenitally determined (Morgan 1896).

Morgan's influence on the early development of an objective, experimental science of animal behavour was profound (see Thorpe 1979), but during the early twentieth century, this embryonic field followed several quite distinct paths. One direction was the highly mechanistic approach of Jacques Loeb. He applied the term "tropism" (used by botanists to describe the bending movements of plants towards light) to invertebrate animals and proposed that their activities and direction of movement were forced on them by their physical and chemical surroundings (Loeb 1918). The rigidity and narrow focus of Loeb's theory (for example, it was of little help in understanding the movements, including long-distance migrations, of vertebrate animals) soon led to its demise. However, it was for a time a useful antidote to the anthropomorphisms of many late nineteenth century students of behaviour. And it may be seen also as an important predecessor to the landmark volume on animal orientation by Fraenkel and Gunn (1940). This was a less mechanistic, more widely applicable approach that was attractive to students of animal movements and orientation in the mid-twentieth century.

Another major development in the early 1900s was the rise of "behaviourism" within experimental psychology in the United States. The key figure in this field was John B. Watson, who argued for hard-nosed, laboratory-based experimental methods when studying animal behaviour (Watson 1914). In later life, he worked almost entirely with humans and adopted an extreme environmentalist position (Watson 1930), which led to a loss of interest in his approach among animal behaviourists, especially in Europe. The early twentieth century also saw the birth of the study of animal learning capabilities, with

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E.L. Thorndike as the most influential figure (Thorndike 1911). As interest in learning increased, this field greatly expanded, especially in the United States, and several general explanatory theories were proposed by, for example, E.C. Tolman, C.L. Hull, and B.F. Skinner.

Meanwhile, during the late nineteenth and early twentieth centuries, there was a strong and continuing interest in understanding animal behaviour by scientists working within a naturalistic tradition. Among the most influential of these were Charles O. Whitman, Wallace Craig, and William M. Wheeler in the United States, Edmund Selous, Julian Huxley, and Eliot Howard in Britain, Oscar Heinroth and Jakob von Uexküll in Germany, and A.F.J. Portielje and J. Verwey in Holland (Thorpe 1979; Dewsbury 1989).

During this early development of a science of animal behaviour (from the 1860s to the 1920s) the animals most often studied were invertebrates (especially some of the social insects), birds, and mammals. The laboratory experimentalists, naturally enough, used species that were easy to maintain in captivity, such as pigeons, doves, mice, and rats. The naturalists/scientists concentrated on species that were easy to observe in nature, such as some social insects, birds, and diurnally active mammals. There is little evidence that fish were either used by experimentalists in the laboratory or studied by naturalists in the field. Difficulty of close observation was probably the main reason for this phylogenetic imbalance in field work; face mask, snorkel, and especially SCUBA were not yet available. Among laboratory workers, even in the United States, where the study of animal behaviour was a young but rapidly expanding field in the early 1900s, fishes were seldom used in experimental work until the late 1920s and 1930s (e.g., Parr 1927; Allee 1931; Breder 1934).

Early research on fish behaviour in Canada

Scientific studies on the behaviour of fishes were also slow to develop in Canada. Even after the establishment of marine research stations on the Atlantic and Pacific coasts in 1908, few scientists appeared to be interested in experimental work on fish behaviour. One of the earliest published accounts was by Philip Cox, working at the St. Andrews Station. He used laboratory and field tests to assess the reaction of adult American eels (*Anguilla rostrata*) to areas of bright light and darkness when they were held in flowing freshwater at night (Cox 1916). Also, A.G. Huntsman, while director of the station, studied the effects of increasing water temperature on survival and respiratory rate of many local fish species (Huntsman and Sparks 1924). Although primitive by later standards, these studies can be seen as early forerunners of the work by F.E.J. Fry and his colleagues (see below) on the influence of environmental factors on the activities of fish.

A major study of routes followed at sea by Pacific salmon (*Oncorhynchus* spp.) on their spawning migrations was conducted over several years, beginning in 1925, at the Pacific Biological Station at Nanaimo. Large numbers of chinook (*Oncorhynchus tshawytscha*) and sockeye salmon (*Oncorhynchus nerka*) were captured by commercial fishing gear, tagged, and released at several locations on the British Columbia coast. Later recaptures of some of these fish produced valuable information on the routes followed by maturing adults (Williamson 1927; Foerster 1968). As part of this research, a pioneering study of the relationship between the olfactory sense and homing ability of salmon was conducted by E.H. Craigie (Craigie 1926). Several hundred sockeye salmon were captured off the northeast coast of Vancouver Island and tagged. Before release, half the fish had their olfactory nerves cut and the others were nonoperated controls. A higher percentage of control than operated fish was recaptured later in the Fraser River, suggesting that an intact olfactory system was essential for effective migration to the fish's home stream (a discovery later confirmed by A.D. Hasler and his colleagues (Hasler and Scholz 1983)). Unfortunately, this promising but inconclusive result by Craigie was not followed up at the time, at least partly because of a lack of scientists in Canada trained in experimental research on fishes (Clemens 1958).

During and after his long tenure as director of the St. Andrews Biological Station and as editor of Canada's main fisheries journal, A.G. Huntsman published many papers on the behaviour of Atlantic salmon (*Salmo salar*). He studied the activities of juveniles in freshwater and the spawning movements of adults as they entered rivers from the sea (Huntsman 1934, 1945). In the absence of data from large-scale adult tagging studies (similar to those begun in the 1920s on the Pacific coast), Huntsman was skeptical that Atlantic salmon migrated to distant feeding grounds at sea and argued that they probably stayed within the influence of water from their "home stream" until they matured, reentered the river, and spawned (Huntsman 1938). However, there was little supporting evidence for his thesis, which attracted few followers.

Soon after the end of the Second World War, fisheries research in Canada entered a period of rapid expansion, supported by the federal government and several of the provinces (Clemens 1952; Fry 1952; Larkin 1952; Johnstone 1977). Much of this effort was aimed at providing a sound basis for sensible management of the commercial and sport fisheries. The study of fish behaviour was not given a high priority at first by those in charge. However, this soon began to change, largely because of the work of two university-based scientists. At the University of Toronto, F.E.J. Fry developed an experimental approach to the analysis of how environmental factors (especially temperature and oxygen content of the water) influence the capacities and activities of fishes. He and his colleagues and students produced a steady stream of influential publications (e.g., Fry et al. 1946; Fry 1947, 1971; Hart 1952; Brett and Alderdice 1958; Brett 1965). Fry's pioneering work had a profound influence on the experimental study of the interactions between fishes and their environment and he soon became a world leader in this field (see Kerr and Lawrie 1976; Evans and Neill 1990; Kerr 1990; Whillans et al. 1990).

At about the same time, William S. Hoar was beginning a career in fish physiology and behaviour at the University of British Columbia. Hoar was primarily interested in the endocrine physiology of migrating salmonid fishes, although an early study that he conducted in Nova Scotia with A.G. Huntsman was on feeding behaviour in young Atlantic salmon and brook trout (*Salvelinus fontinalis*) (Hoar 1942). Shortly after moving to British Columbia, Hoar had the good fortune to meet Niko Tinbergen, one of the founders of modern ethology, a new approach to the study of animal behaviour pioneered in the 1930s and 1940s in Europe (see below). In 1947, Tinbergen was invited to visit the University of Alberta by William Rowan, one of the first investigators in Canada of bird

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migration, and then went on to the University of British Columbia. He suggested to Hoar that the five species of Pacific salmon presented an excellent opportunity for a comparative study of behaviour at the time of their seaward migration (W.S. Hoar, personal communication). Beginning in the 1950s, Hoar and his students developed an impressive research program into the social and migratory behaviour of juvenile Pacific salmon (e.g., Hoar 1951, 1953, 1958; Hoar et al. 1952; Keenleyside and Hoar 1954). Hoar went on to become an international leader in the study of fish physiology and behaviour (see Randall et al. 1982).

Thus, with Fry and Hoar leading the way, a new generation of scientists trained in the experimental study of fish behaviour began to influence the way in which fisheries research was being done in Canada. At the same time, the science of animal behaviour was rapidly developing throughout the world, especially under the influence of European ethology.

Influence of ethology on animal behaviour research

Although ethology as a distinct approach to the study of animal behaviour arose in the nineteenth century (Jaynes 1969; Thorpe 1979), it did not begin to dominate the field until the 1950s and 1960s. Its most influential pioneers were Karl von Frisch, Konrad Lorenz, and Niko Tinbergen. The first two were Austrian-born, but spent much of their working lives in Germany; Tinbergen was Dutch and moved to England part way through his career. All three shared the 1973 Nobel Prize in physiology or medicine for their contributions in animal behaviour.

von Frisch is best known for his discovery that honeybees use an elaborate communication system in the hive to direct foragers to a newly discovered food source (von Frisch 1950). But he also did pioneering work with fishes, including their visual, auditory, and chemical senses. Above all, he discovered the alarm pheromone ("Schreckstoff") phenomenon in cyprinids (von Frisch 1941), which opened up the field of chemical communication in fishes (Smith 1992). Konrad Lorenz is generally considered to be the key figure in the emergence of European ethology. He was a superb naturalist/observer and was remarkably skilled at rearing animals in semicaptivity (i.e., tame but free to move at will). This helped him to begin to understand the relationship between behaviour patterns that were largely inherited and those that were influenced by experience early in life (Thorpe 1979). He found the other major approaches to the study of animal behaviour (such as Gestalt psychology, reflexology, vitalism) to be seriously deficient and, as a reaction to them, developed his own general theory, which became the foundation of modern ethology (Beer 1963–1964). Lorenz published a series of lengthy papers in the 1930s, several of which have been translated into English (Schiller 1957). He had a powerful, charismatic personality and was an excellent raconteur (see his "King Solomon's ring" (Lorenz 1952)), but he was not an experimental scientist. It was therefore ethology's good fortune that he soon began to collaborate with Tinbergen (Lorenz and Tinbergen 1938). Not only was Tinbergen another skilled naturalist, he was also gifted at designing simple but elegant experiments to answer research questions, especially in the field. In addition, he stressed the advantages of a broad approach to the understanding of behaviour. Research should explore the causation, function, development, and evolution of behaviour patterns. And whenever possible, the work should be done against a solid background of knowledge of the species' natural environment (Tinbergen 1963).

With the creative examples set by Lorenz and Tinbergen, ethology soon began to attract many young biologists in continental Europe. When Tinbergen moved from Leiden to Oxford in 1949 and then published "The study of instinct" in English (Tinbergen 1951), the new ethology quickly spread into the English-speaking world (Dawkins et al. 1991). Both Tinbergen and Lorenz made several trips to North America to describe their approach, especially at universities, where students found it attractive (Dewsbury 1989). The founding of *Behaviour: an International Journal of Comparative Ethology* in 1948, published in Holland with an international board of editors, including two from the United States, helped to establish the field. W.H. Thorpe of Cambridge University contributed to the wider understanding of ethology by organizing a symposium of the Society for Experimental Biology, held at Cambridge in 1949. The meeting was titled "Physiological mechanisms in animal behaviour" and it brought together leading ethologists and physiologists interested in animal behaviour.

The spread of European ethology to Canada was also promoted by Canadians who went to study with pioneering ethologists and returned to take up positions in government research laboratories or universities, where their training and experience were passed on to the next generation. For example, M.H.A. Keenleyside received his Ph.D. in 1955 at the University of Groningen, under G.P. Baerends, one of Tinbergen's earliest students, and P.E.K. Symons obtained his Ph.D. in 1965 at the University of Leiden, with J.J.A. van Iersel, a close colleague of Tinbergen's. In addition, several Europeans who were trained in the laboratories of leading ethologists came to Canada as postdoctoral fellows in the 1950s and 1960s. These included W. Pfeiffer, who had studied with von Frisch at Munich, B. Baggerman, from Baerends' laboratory in Groningen, C. Groot from van Iersel's laboratory in Leiden, N.R. Liley and J.N.M. Smith from Tinbergen's group at Oxford, and J. Fentress from W. Thorpe's laboratory at Cambridge. Many of these early converts to the methods and principles of ethology used fishes as their experimental animals; thus, fish behaviour research was especially enhanced by the new ethology. In a more detailed review, Dewsbury (1995) has described the spread of ethology from Europe to the United States and Canada before 1975 by documenting the North American behaviourists who went to Europe to study with ethologists and the European ethologists who moved to work in North America.

A continuing source of contact between Canadian behaviourists and European ethologists have been the International Ethological Conferences. The first was a small meeting in 1950 in Wilhelmshaven, Germany, with Erich von Holst as convenor. He was a physiologist whose work provided the basis for Lorenz's early ideas on the internal controls of behaviour (Lorenz 1950). This gathering was so productive that another was held at Lorenz's institute at Buldern, Germany, in 1952 and then another at Oxford in 1953. Since then, they have been held in alternate years at centres of behavioural research around the world. The only International Ethological

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Fig. 1. Total number of papers (circles) and the number of those papers dealing with fish behaviour (triangles) published (A) from 1901 to 1994 in CJFAS and its predecessors and (B) from 1935 to 1994 in CJZ. The fish behaviour curves include only papers whose authors used a Canadian address. Data are grouped by 5-year intervals.

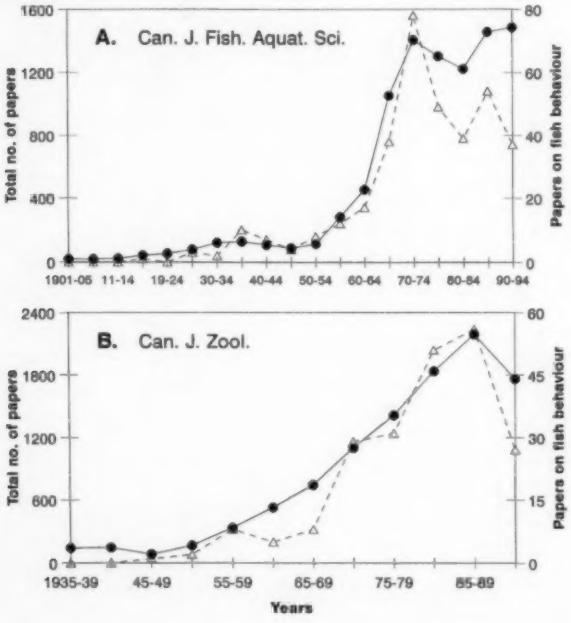
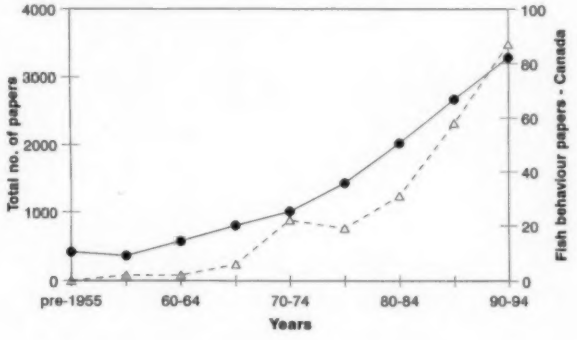


Fig. 2. Total number of papers (circles) and the number of those papers dealing with fish behaviour and whose authors used a Canadian address (triangles) published in six animal behaviour journals. Data are grouped by 5-year intervals. Journal titles and the year each began publishing are given in the text.



Conference in Canada was in 1979 in Vancouver, where N.R. Liley of the University of British Columbia was the chief local organizer.

In Canada, following the pioneering work of Fry and Hoar, and with the enthusiastic adoption of ideas from the new ethology, the experimental study of fish behaviour soon became established in several universities and government research laboratories. There was some resistance to the ethological approach at first, especially by animal physiologists, because many of the early studies (in the 1930s and 1940s) lacked adequate experimental controls, large sample sizes, and rigorous data analysis. Also, the emphasis by European ethologists on the concept of instinct was irritating to North American zoologists, most of whom by the mid-twentieth century believed that instinct was a poorly defined, fuzzy label and certainly not one to be used as an explanation for poorly understood behaviour. However, as it became clear that the term was meant to be applied to species-specific behaviour patterns that appeared to be largely under genetic control, and were especially common among insects, fishes, and birds (the favourite study animals of early ethologists), this criticism of ethology subsided.

Then in 1975, with the publication by E.O. Wilson of "Sociobiology: the new synthesis" (Wilson 1975), the study of animal behaviour took a new turn. This book was a masterful synthesis of information on behaviour, ecology, and population biology, all set within an evolutionary explanatory framework. However, it soon became controversial because in the final chapter,Wilson extended his thesis to include the human species. The debate became largely political because of the implication (to some critics) that much of human behaviour is determined by our evolutionary history and therefore resistant to change (Barlow 1991). Most animal behaviourists soon tired of this increasingly sterile debate and returned to their work in the field and laboratory.

The experimental approach that has come to dominate much of animal behaviour research since Wilson's pioneering synthesis in 1975 is behavioural ecology, which has been described as the study of the survival value of behaviour (Krebs and Davies 1993). Emphasis is on learning how animals acquire resources (such as food, shelter, and mates) while avoiding predation. The study of "decision-making" by individual animals faced with often conflicting requirements has become a central feature of the field (Dill 1987). Of course, there are other areas of special interest within modern animal behaviour research. The study of physiological controlling mechanisms, migratory behaviour, rhythms, communication, and others is all part of a vigorous and still-expanding discipline. However, behavioural ecology currently seems to be the most dynamic area within the broader field. In 1990 the journal *Behavioural Ecology* was founded, with an international board of editors. Canadian scientists, including many working with fishes, have been active contributors to this and the older, more established journals of animal behaviour.

Development of Canadian research on fishes

The best indicator of the level of activity in a scientific field is the number of publications in peer-reviewed journals in that field. Of course, the quality of the research, as assessed, for example, by the influence those papers have on other workers, is also of great interest. However, I deal here only with quantitative aspects of the development in Canada of fisheries research in general and fish behaviour research in particular. To do this, I have used two approaches. First, I present data on the numbers of papers published in selected journals over many years. Second, I show the changes over time in the species studied and the types of behaviour investigated.

To illustrate the changes in fisheries research publications

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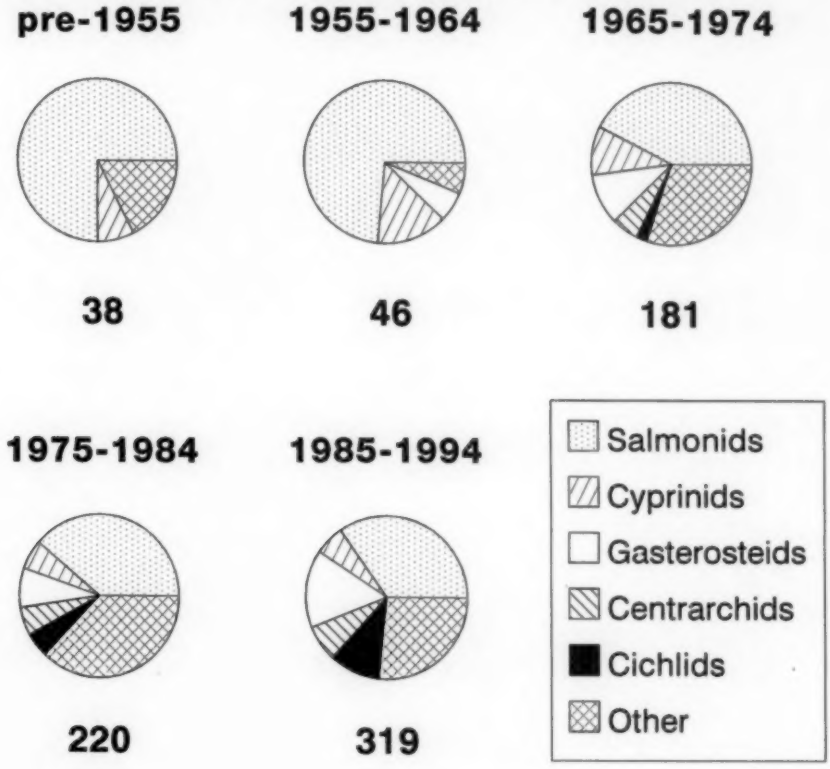
Fig. 3. Fishes used in Canadian fish behaviour research. Data are from CJFAS, CJZ, and six specialty journals. The circle for each decade (all pre-1955 data pooled) shows the percentage of fish used that are in the five most commonly used families (all others grouped as "Other"). The number under each circle is the total number of Canadian-based papers on fish behaviour for that period.



in Canada, I first chose the present journal (*Canadian Journal of Fisheries and Aquatic Sciences* (CJFAS)) and its predecessors. There have been four name changes since Volume 1 was published in 1901 with the title *Contributions to Canadian Biology*. For each year's volume, I read the list of titles and if any suggested that fish behaviour was included, I read the abstract or the entire paper. If behaviour was an important part of the content, I scored it as a fish behaviour paper, even if nonbehavioural material was included. Since the main focus of this paper is on fish behaviour research in Canada, I included in this tally only those papers whose authors used a Canadian address. Although the CJFAS has become a truly international journal and many of its papers are based on work done outside Canada (especially the United States), I used the total number of papers in each volume, regardless of author's address, as an indicator of the overall strength of this field of research. In no year was the proportion of papers with Canadian-based authors less than 50%.

Until the mid-1950s the total number of papers and the number of those dealing with fish behaviour in CJFAS were consistently low (Fig. 1A). Then there was a rapid increase in both categories, reaching peaks in 1970–1974. This increase reflects the rapid rise in the amount of fisheries research in Canada following the Second World War. The total of all papers declined slightly over the next decade, but has been increasing since then. The number of fish behaviour papers decreased sharply after 1970–1974 and has since been irregular. The decline in both curves after the mid-1970s may be related to a change in the journal's editorial policy in 1976. To cope with steadily rising publication costs and manuscript submission rates, the editors announced that purely descriptive papers, and especially those based on "case history studies," would no longer be accepted (Stevenson 1976). This no doubt had some effect on manuscript submissions, and perhaps especially on those dealing with behaviour, because descriptive studies are common in the behaviour literature. Another statement of editorial policy, published in 1983 (Watson and Reinhart 1983), may also have discouraged the submission of manuscripts by behaviourists.

However, there is another possible explanation for the decline in fish behaviour papers in CJFAS after 1974. Two new journals in the field began publication in 1976. One of them (*Behavioral Ecology and Sociobiology*) was published in Germany, but the other (*Environmental Biology of Fishes*) has its editorial offices in Guelph, Ontario, and from its inception has published papers dealing with fish behaviour. Thus, some manuscripts were probably directed by Canadian workers to these two journals rather than to CJFAS.

I followed the same scoring procedure with the *Canadian Journal of Zoology* (CJZ), which was titled Section D of the *Canadian Journal of Research* from 1935 to 1950. Even though a high proportion of authors in this journal were based

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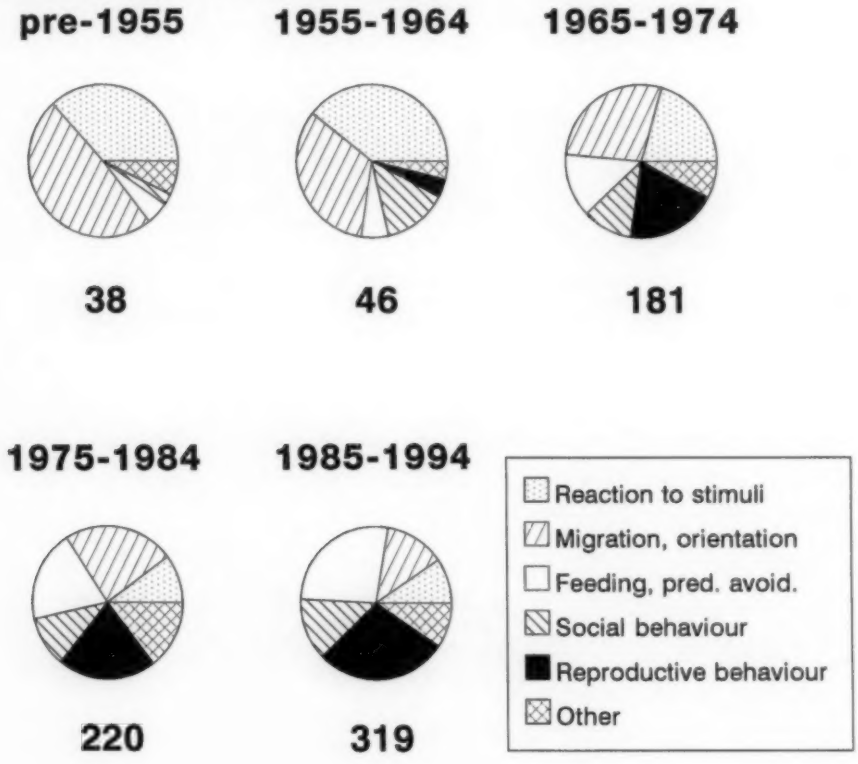
Fig. 4. Categories of behaviour studied in Canadian fish behaviour research. Data are from the same sources as in Fig. 3. Each circle shows the percentage of topics studied that are in the five most commonly studied topics (all others grouped as "Other"). The number under each circle is the total number of Canadian-based papers on fish behaviour for that period.



in Canada, a few were not. To conform with my tally system for CJFAS, I excluded from the CJZ fish behaviour scores the few papers whose authors did not use a Canadian address, but included these in the total for each volume. As with CJFAS, the publication rates of all papers and those on fish behaviour were low until the mid-1950s (Fig. 1B). The total number then increased steadily to a peak in the late 1980s, followed by a slight decline. The fish behaviour curve shows a similar but less regular increase, and a decline in the early 1990s. The latter may have been influenced by the founding of *Behavioral Ecology* in 1990, a journal in which eight fish behaviour papers from Canadian laboratories were published from 1990 to 1994.

A study of fish behaviour research in Canada would be incomplete without including data from the specialty animal behaviour journals. I chose the six most important journals in the field, which are, with the first year of publication, *Ethology* (formerly *Zeitschrift für Tierpsychologie*) (1937), *Behaviour* (1948), *Animal Behaviour* (formerly *British Journal of Animal Behaviour*) (1953), *Environmental Biology of Fishes* (1976), *Behavioral Ecology and Sociobiology* (1976), and *Behavioral Ecology* (1990).

For each volume of the six journals, I tallied the total number of papers and the number dealing with fish behaviour whose authors were based in Canada (Fig. 2). There is a steady increase in total publications in these journals from 1950 to 1994, reflecting the rising interest in animal behaviour research throughout the world since the immediate postwar period. The Canadian-based publications in fish behaviour in these journals did not begin to increase until the late 1960s, and from 1975 onward there is a steep upward slope to the curve (Fig. 2).

To document the changes during this century in the fish species used by behaviourists in Canada, I examined all the papers on fish behaviour that I had used for Figs. 1 and 2. I grouped the species into the five most commonly used families and a single "Other" category.

Salmonids were by far the most commonly used research species until the mid-1960s, but since then have declined to about one third of the total (Fig. 3). This presumably reflects their dominant position in commercial fisheries of the Pacific coast, and of sport fisheries throughout Canada. The decline in relative frequency of salmonids in the behaviour literature beginning in the mid-1960s corresponds to the rapid increase in fish behaviour research at about that time (Figs. 1 and 2). As the use of fishes in behaviour research became more popular in Canada, the range of study species broadened to include many nonsalmonids that are easy to maintain and breed in the laboratory. Also, by this time, snorkelling and SCUBA had made it possible for behaviourists to observe and to run experiments with many types of fishes in nature.

Gasterosteids have occupied a small but consistent proportion of studies since the 1950s (Fig. 3). They are small, easy

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to maintain in the laboratory, and some species are easy to observe in nature. The threespine stickleback (*Gasterosteus aculeatus*) has become a famous behavioural symbol because it was prominent in early European ethology (Tinbergen and van Iersel 1947; Tinbergen 1951). This single species continues to be of great interest to students of behaviour, ecology, and evolutionary biology (see Bell and Foster 1994). Centrarchids and cyprinids, both of which are widespread in Canada, have each ranged from 5 to 15% of species used since about midcentury, again reflecting the small size and ease of maintenance of at least some species in these families. Cichlids have increased from a tiny fraction of species used in 1965–1974 to about 10% recently (Fig. 3). This large family of tropical and subtropical, freshwater fishes has no species native to Canada, but some can be studied in nature and many are well suited for laboratory research (Keenleyside 1991).

I also examined the variation in the particular aspect of behaviour that was studied by fish behaviourists in Canada. Using the same literature sources as for Fig. 3, I assigned each paper to one (rarely two) of six categories of behaviour (Fig. 4). Some categories include a range of behaviour patterns. For example, under migration and orientation, I included studies on performance (such as swimming speed) and activity. The "Other" category includes any topic not clearly assignable to the other five (for example, habitat selection and local movements, as opposed to large-scale migrations). Also, even though reproduction is clearly a social activity, I placed it in a separate category because it is such a distinctive and critical form of behaviour. I combined feeding and predator avoidance because in recent years, much experimental work has centred on the interrelationships between these two activities.

Over 85% of the studies before 1955 fell into the first two categories, that is, reactions to environmental stimuli and migration/orientation (Fig. 4). Work in these two still predominated during 1955–1964 (73%), but declined steadily after that. This supports the observation that these were two of the areas in fish behaviour where Canadian investigators pioneered, but that were later replaced as newer approaches were developed for the analysis of behaviour, first in ethology and then in behavioural ecology.

A modest but steady proportion of the work since 1955 has been on various aspects of fish social behaviour (including schooling and territorial and dominance behaviour). In contrast, the effort directed at reproductive behaviour has steadily increased from less than 5% at midcentury to 28% of the total at present. The other category showing steady growth is that of feeding/predator avoidance. Analysis of the interrelationships between these two activities appears particularly suitable for experimental work (Dill 1987), which may explain its frequency.

Almost 100 years ago the Canadian government committed itself to the public support of scientific research as an essential tool for the understanding of both fish and fisheries. That support, which expanded greatly after midcentury, has resulted in Canada becoming a world leader in fisheries science. At the close of the twentieth century, research on the behaviour of fishes has come to occupy a small but vigorous part of that science. At the same time, some of our major commercial fisheries are once again in serious trouble; those based on northern cod and some Pacific salmon stocks are the most spectacular examples. Is there any reason to suppose that fish ethologists and behavioural ecologists can contribute to solving such problems, when persistent overfishing and habitat degradation appear to be the major offenders?

Historically, fish behaviourists in Canada have concentrated on advancing basic knowledge, rather than helping to solve management problems. However, there are signs that this attitude may be changing. At the 1996 meeting of the Canadian Conference for Fisheries Research, a symposium was held on the topic "Behavioural ecology and fisheries science." Papers in that session dealt with behavioural consequences of salmonid captive breeding programs, habitat selection and the design of fish reserves, and fishing vessels as predators. The value of applying insights from the field of animal behaviour to the solution of problems in Canada's fisheries has yet to be established. However, the initial signs are encouraging.

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DISCUSSION

Comment: Toward a definition of conservation principles for fisheries management[1]

Stephen S. Crawford and Bruce Morito

More than a year ago, Olver et al. (1995) published their perspective on the definition of conservation principles for fisheries management. In their article, the authors discussed three aspects of conservation, within the general context of fisheries management:

(1) the North American history of European-based conservation ethics,
(2) their proposal for a definition of the term 'conservation,' and
(3) their proposal for seven conservation principles for use in fisheries management.

In their concluding remarks, Olver et al. (1995, p. 1592) state:

> "Our intent... is to promote an ecological ethic as the foundation for developing a set of conservation principles for the management of fisheries resources in Canada, and to present a set of conservation principles for fisheries management that will evoke a debate about just what those principles ought to be. With respect to the former, we would expect general support from the fisheries community, but we are under no illusion that the fisheries community will endorse this preliminary statement of conservation principles. Any subsequent debate that this article may spark will, however, contribute to a further refinement and elucidation of such principles."

Olver et al. (1995) are among the first Canadian fisheries scientists to propose a conservation ethic for fisheries management. We support this undertaking. We agree with their suggestion that Canadian fisheries are in urgent need of an effective conservation ethic that will guide future policy development and management activities (Callicott 1991). In support of their objectives and their explicit request for further debate on this topic, we wish to respond to their proposal by identifying three aspects of the conservation ethic.

The founding role of explicit values in a conservation ethic

Within the discipline of applied ethics, an ethic is an integrated system of values, principles, and judgements that determines the difference between right and wrong in human behaviour (Beauchamp and Childress 1979; Thomas 1983; Regan 1984). Within such a system, values are motivational factors that dispose people to act. Principles are guiding factors, formulated as general rules of conduct, that promote the satisfaction of particular values. Judgements are deciding factors; the specific applications of a general principle. Ranking principles, in turn, depends on the weighting of values upon which they are based. If one wishes to define an ethic, one must attempt to determine and rank the underlying values, articulate the principles, and establish the specific rules for making judgements.

Olver et al. (1995) do not explicitly identify the values underlying their conservation ethic. As a result, the reader must try to isolate and interpret implicit references to founding values in the text. For example, in different places the authors apparently make reference to some of their values by using phrases such as "biodiversity," "continuity of ecosystem processes," "ecosystem sustainability," "ecosystem integrity," "ecosystem health," and "healthy life-support systems." If these are in fact references to values underlying Olver et al.'s (1995) conservation ethic, then the reader faces the task of determining what these values actually are. Although phrases such as ecosystem integrity and ecosystem health sound appealing, several other authors have warned about problems with such concepts, because they are often ill defined and they may be based on invalid theoretical foundations (e.g., Suter 1993; Callicott 1995; Wicklum and Davies 1995; Ghilarov 1996). Without a full and clear explanation of what Olver et al. (1995) mean by these phrases, the possibility exists for development of inconsistent principles and judgements. For this reason, we see the need for further debate on the values underlying the proposed conservation ethic that Olver et al. (1995) have initiated.

The effect of ambiguous values extends into other aspects of Olver et al.'s (1995) conservation ethic. For example, consider the authors' definition of the term "conservation" (Olver et al. 1995, p. 1587):

> "A conservation ethic based on ecological values requires

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[1] Paper by C.H. Olver, B.J. Shuter, and C.K. Minns. 1995. Can J. Fish. Aquat. Sci. **52:** 1584–1594.

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> that humans derive social, economic, recreational, and cultural benefits in a sustainable manner. A definition of conservation consistent with this position follows: the protection, maintenance, and rehabilitation of native biota, their habitats, and life-support systems to ensure ecosystem sustainability and biodiversity."

We recognize two general problems with this definition. First, it appears to us that Olver et al. (1995) attempt to define the term conservation by combining some of the ill-defined values discussed above. Second, and more importantly, the wording of Olver et al.'s (1995) definition of conservation is so general that it would be difficult to employ it as an operational tool (see Peters 1991). Imagine the difficulty in trying to determine whether a particular fisheries management action would be consistent or inconsistent with the definition of conservation presented above. For this reason, we suggest that it would be more useful to work on defining the structure and function of a conservation ethic, rather than to define the actual term conservation.

The absence of clearly defined values in the article by Olver et al. (1995) also makes it impossible to determine whether their set of proposed conservation principles are actually coherent and consistent with their founding values (i.e., do the principles promote the satisfaction of a particular value?). We strongly urge Olver et al. (1995) and others, to revisit the task of explicitly identifying the values held by fisheries biologists with respect to conservation. What motivates their interest and understanding in conservation as it applies to fisheries? Why is this an important subject of development and debate in their discipline? By answering these kinds of questions, they should be able to define biological values in a manner that facilitates greater understanding of the connection between values and principles. We realize that such an undertaking is no small task; however, we consider this to be a primary requisite for the development of an effective conservation ethic.

Essential biological objectives for a fisheries conservation ethic

If we assume that Olver et al. (1995) will be successful in articulating the conservation values of fisheries biologists, what happens then? Will it simply be a matter of using the newly defined biological values to construct a conservation ethic by evaluating, selecting, and refining principles that promote the satisfaction of those biological values? This seems to be the approach adopted by Olver et al. (1995, p. 1585) in their paper:

> "Our concern, shared by Parsons (1993), is that the lack of a clear definition of conservation may foster misconceptions about the degree to which biological objectives can be safely traded off against pressing economic and social objectives. Our purpose is to promote a dialogue about the meaning and practice of conservation, in the hope that this will lead toward consensus on *essential biological objectives* [our emphasis]."

Clearly, the authors believe that it is desirable to establish the primary constraints of a fisheries conservation ethic in biological terms, thus setting the bounds within which economic and social issues may further shape fisheries management decisions. These primary constraints would effectively establish the minimum criteria of biological conditions for fisheries management that could not be "traded-off" under any circumstances. At first glance this appears to be perfectly reasonable; however, closer consideration of the argument raises two important concerns.

First, we must ask what biological objectives for fisheries management are, and how these differ from social and economic objectives. Although this issue is at the heart of Olver et al.'s (1995) paper, the authors do not clearly distinguish between the different kinds of objectives for fisheries management (see Hilborn and Walters 1992). To demonstrate the difficulty of this question, we would ask the reader to reflect on what is meant by biological objectives. When we refer to biological objectives are we talking about the interests of the fish populations that would support a fishery or perhaps the ecosystem (however defined) that would support the fish populations, or are we talking about the interests of humans who seek to benefit from the fishery? Such fundamental questions have profound ramifications on what might be meant by the phrase essential biological objective.

A second major concern with Olver et al.'s (1995) essential biological objectives has to do with the practicality of focussing exclusively on biological factors to establish the primary constraints of a conservation ethic (Mangel et al. 1996). Is it desirable, or even possible, to establish biological objectives (however defined) without consideration of social or economic factors? Are biological objectives themselves not a form of social or economic objective? Olver et al. (1995, p. 1586) implicitly recognize the difficulty in separating these factors when they refer to "the concept of the ecosystem approach (Christie et al. 1986), which aims to balance ecological, economic, and social objectives and recognizes that human uses must be reconciled with intrinsic and necessary functions and structures." In their proposal, the authors proceed with the development of a conservation ethic as if it were possible to identify and define essential biological objectives.

This predicament leads to further difficulties when Olver et al. (1995) propose their fisheries conservation principles. Consider, for example, the difficulty Olver et al. (1995, p. 1591) encounter when discussing their biological objective regarding direct exploitation of spawning aggregations:

> "The west coast anadromous stocks spend much of their life cycle in oceanic habitats so large that efficient harvesting is not possible. Only when they form natural aggregations, just before spawning, are they sufficiently concentrated in space to be effectively harvested. The abundance and economic value of these stocks justify large expenditures for the assessment and enforcement activities necessary to restrict spawner-targeted harvests to levels where the risk to sustainability is acceptable.
>
> Among freshwater stocks, only a tiny percentage of the millions of discrete stocks scattered throughout the lakes and rivers of Canada are of sufficient economic importance to support the information-intensive, micromanagement practices demanded by spawner-targeted harvesting."

Clearly, the authors have taken socioeconomic considerations into account as a primary constraint in the development of this principle. In this case, Olver et al. (1995) argue that the harvest of spawning fish is acceptable, if and only if the economic value of that harvest is sufficiently great to justify the application of appropriate stock assessment methods. Have the

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authors not, in fact, delivered a biological objective that is already traded off against economic concerns?

Stakeholder values on essential biological objectives

It should be clear that Olver et al. (1995) framed the development of their conservation ethic within the context of Western biological science. While we do not question the necessity of incorporating Western biological values into a modern fisheries conservation ethic, we do question whether such a context is sufficient for definition of essential biological objectives. It would be naive to suggest that biologists are the only stakeholders in Canadian fisheries that hold values related to biological objectives for fisheries conservation. Within the context of Canadian fisheries, the potential stakeholder groups would include the following broad categories: (*i*) Aboriginal communities (First Nations), (*ii*) commercial fisheries, (*iii*) recreational fisheries, (*iv*) environmental protection organizations, (*v*) fisheries biologists, (*vi*) fisheries managers, and (*vii*) the general public. While each of these groups may have social or economic interests in a particular fishery, they may also hold biological values for fisheries conservation that are not held by most fisheries biologists. While the conservation ethic proposed by Olver et al. (1995) may provide a good fit with the biological values of some fisheries interest groups, the authors did not explicitly attempt to incorporate the biological values of other major stakeholder groups in their conservation ethic. According to recommended fisheries management practices, any important management action (biological or otherwise) requires consultation and meaningful consideration of major stakeholders' values (Felt 1990; Decker and Krueger 1993; Ballweber and Jackson 1996). A possible consequence of not considering the biological values of stakeholders is to marginalize those people and to unjustly impose a set of rules on a group that holds dissimilar values (Peyton 1987).

We are particularly concerned that Olver et al. (1995) developed their conservation ethic without any consideration of Aboriginal values or perspectives on fishes and fisheries. We consider this omission to be particularly noteworthy, given the authors' own reference to the Regina versus Sparrow (1990) case at the outset of their paper. The Sparrow case is a landmark decision of the Supreme Court of Canada, which establishes several precedents for the management of fisheries in this country (Usher 1991) including the need for meaningful consultation with Aboriginal communities on issues related to conservation:

> "Within the analysis of justification, there are further questions to be addressed, depending on the circumstances of the inquiry. Those include the question of ... whether the Aboriginal group in question has been consulted with respect to the conservation measures being implemented." (Regina vs. Sparrow 1990, p. 34.)

More recently, Regina versus Van der Peet (1996), along with the companion appeals in Regina versus Gladstone (1996) and Regina versus N.T.C. Smokehouse (1996) raise an important issue left unresolved in Sparrow; the manner in which Aboriginal rights, recognized and affirmed by section 35(1) of the Constitution Act (1982) of Canada, are to be defined:

> "... the doctrine of Aboriginal rights exists, and is recognized and affirmed by s.35(1), because of one simple fact: when Europeans arrived in North America, Aboriginal peoples were already here, living in communities on the land, and participating in distinctive cultures, as they had done for centuries. It is this fact, and this fact above all others, which separates Aboriginal peoples from all other minority groups in Canadian society and which mandates their special legal, and now constitutional, status. More specifically, what s.35(1) does is provide the constitutional framework through which the fact that Aboriginals lived on the land in distinctive societies, with their own practices, traditions and cultures, is acknowledged and reconciled with the sovereignty of the Crown." (Regina vs. Van der Peet 1996, p. 48.)

Thus, according to the Supreme Court of Canada, Aboriginal communities represent a special class of fisheries stakeholder; this distinction is based on the fundamental difference between the legal concepts of "rights" versus "privileges" (Turpel 1991).

In our opinion there is a clear ethical requirement for Olver et al. (1995) and others to take special care in considering Aboriginal perspectives on fisheries conservation. However, we should caution that there is tremendous potential for differences in the perspectives of Aboriginal and non-Aboriginal stakeholders on the roles of science and technology in determining conservation principles for "resource management" (Gamble 1986, Deloria 1995; see also Dwyer 1994). Incorporating stakeholder values into an ethic regarding fisheries conservation is going to require substantial effort; incorporating Aboriginal values on this matter will be a challenge (e.g., Freeman 1985; McDaniels et al. 1994).

Future development of a conservation ethic for fisheries management

Based on our discussion of issues raised by Olver et al.'s (1995) proposed conservation ethic, we would like to pose three general questions regarding future development on this topic.

(1) How can we provide a more complete explanation of the founding biological values and the manner in which conservation principles promote these values?
(2) How can we distinguish between essential biological objectives and social or economic objectives for fisheries management, and is it possible to establish a conservation ethic solely on essential biological objectives?
(3) How do we propose to incorporate other stakeholders' values in the establishment of a conservation ethic for fisheries management?

It is our hope that these questions continue the constructive debate on the conservation ethic for fisheries management initiated by Olver et al. (1995).

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Reply: Toward a definition of conservation principles for fisheries management[1]

B.J. Shuter, C.K. Minns, and C.H. Olver

We thank Crawford and Morito for their comments regarding our essay on conservation principles (Olver et al. 1995). We will focus our reply on the three questions that Crawford and Morito pose at the close of their commentary. The views we express are ours alone. We do not presume to speak for all fisheries biologists nor are we promulgating the policies of our respective agencies.

Question 1: How can we provide a more complete explanation of the founding biological values, and the manner in which conservation principles promote these values?

We think that our definition of conservation contains a relatively clear statement of the ecological value that underlies our definition: native ecosystems are valued; it is "wrong" to act in such a way as to impair their intrinsic ability to persist through time. Hence, conservation is "the protection, maintenance and rehabilitation of native biota, their habitats and life support systems to ensure ecosystem sustainability and biodiversity." We note that our definition of conservation agrees almost exactly with Mangel et al's (1996) second principle for the conservation of wild living resources: "The goal of conservation should be to secure present and future options by maintaining biological diversity at genetic, species, population and ecosystem levels; as a general rule neither the resource nor the other components of the ecosystem should be perturbed beyond natural boundaries of variation."

We dispute the contention that ecosystem sustainability and biodiversity are "ill-defined values." These terms do not refer to "values" but rather to valid ecological concepts that can be operationally defined. Useful explanations of both terms are given in Mangel et al.(1996); however, we prefer the following:

(1) Grumbine's (1994) clear prescription for maintaining ecological integrity, which we take to be synonymous with the requirements for ecological sustainability: maintain viable populations of native species in situ; maintain the representation of native ecosystems within their biogeographic range; maintain evolutionary and ecological processes, and hence maintain the evolutionary potential of species and ecosystems.

(2) Noss' (1990) account of the ways in which biodiversity is expressed across fours levels of ecological organization: the landscape, the community, the population, and the individual through its genotype.

We dispute the contention that our definition of conservation is too vague to be of any use. Our definition, much like Mangel et al's (1996) second principle, states the goal of conservation, and the goal is clear. In our paper, actions that we thought could operationalize this goal were outlined in our accompanying principles. This contrasts with Mangel et al. (1996), who chose to include both the "means" and the "ends" of the conservation enterprise in a single list of principles. We believe it is useful to separate ends from means, since we contend that consensus among stakeholders on goals is both possible and desirable, while consensus on means may be a much greater challenge.

Question 2: How can we distinguish between "essential biological objectives" and "social or economic objectives" for fisheries management, and is it possible to establish a conservation ethic solely on essential biological objectives?

We think that it is possible to base a conservation ethic solely on essential biological objectives. We agree with Mangel et al. (1996) that the goal of conservation is to ensure that existing ecosystems continue to persist through time. As biologists, we see this goal in terms of the purely biological requirements that ecological persistence demands. The conditions that satisfy these requirements should set the bounds within which economic and social issues are allowed to shape management decisions. Like Grumbine (1994), we argue strongly for a two-stage process: biological considerations establish constraints on overall resource use; within those constraints, legal rights and socioeconomic concerns determine the level of use and how that use is shared among stakeholders. A two-stage process is critical because (*i*) it reinforces the primacy of conservation requirements over resource use, and (*ii*) it clearly

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separates issues that are relevant in determining constraints on overall use from issues that are relevant in allocating allowable use among stakeholders.

Question 3: How do we propose to incorporate other stakeholders' values in the establishment of a conservation ethic for fisheries management?

We are optimistic that other stakeholders will accept our contention that the primary goal of fisheries conservation is to ensure the continued existence of native ecosystems and their component fish populations. We recognize that there will be much disagreement regarding the best means to achieve this end. We presented our original set of supporting principles as a preliminary list of means; our hope was to generate comment and debate, the raw materials needed to develop a working consensus or modus vivendi among stakeholders.

We recognize that many of our original principles implicitly assume that resource use is intense and driven solely by the desire to maximize biomass caught, which is the traditional objective of a commercial fishery. Such principles are needed because many of the world's large commercial fisheries are being overexploited now (e.g., Myers et al. 1997), as are many intense, consumptive recreational fisheries (Evans et al. 1991). However, for subsistence fisheries, ceremonial fisheries, and nonconsumptive recreational fisheries, very different motivations drive use, and hence, such fisheries may require quite different supporting principles. With respect to the means needed to effectively meet the goals of conservation, we strongly support Mangel et al.'s principle 6 (effective conservation requires understanding and taking account of the motives, interests, and values of all users and stakeholders but not by simply averaging their positions) and principle 7 (effective conservation requires communication that is interactive, reciprocal, and continuous). These principles are echoed in the Ecological Society of America's report on the science of ecosystem management (Christensen et al. 1996), and in FAO's guidelines for ethical fisheries (FAO 1995).

In fishery management and the wider arena of ecosystem management, we think increased efforts to develop and implement operable conservation principles are essential, because fisheries and ecosystems are threatened by human actions everywhere. We hope that the comments of Crawford and Morito, along with our reply, will stimulate greater attention to this issue among all stakeholders.

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